Spatial and Temporal Dynamics of Phytoplankton Communities in a Coastal Ecosystem

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ABSTRACT

The phytoplankton community dynamics and the processes influencing phytoplankton community structure were investigated in Beatrix Bay, Pelorus Sound, New Zealand. The particular focus was on bottom-up resource acquisition driving phytoplankton dynamics in this coastal ecosystem. Sampling for water column structure, nutrient concentration, and phytoplankton community composition was done over two years in conjunction with experiments that manipulated nutrient concentration and light levels to test how these affected phytoplankton community dynamics seasonally.

The water column was thermally stratified during summer. During the rest of the year stratification was salinity-driven and related to the level of prior freshwater input from the Pelorus River, the main source of freshwater to Pelorus Sound. Phytoplankton in Beatrix Bay tend to be light-limited during winter. Nitrate was limiting to phytoplankton growth during spring and summer, when ambient nitrate levels were consistently below 0.5 μM. Small to medium sized, chain-forming diatom taxa such as *Chaetoceros* sp., *Pseudonitzschia* sp., and *Skeletonema* sp., were the taxa that responded most to nitrate enrichment in experiments. These are the taxa that dominate Beatrix Bay biomass during most of the year. This phytoplankton assemblage is characteristic of ecosystems that receive frequent inputs of nutrients. Dinoflagellates tend to only attain high biomass during summer, when the water column is thermally stratified and nitrate levels are depleted.

Interannual variability in phytoplankton community dynamics was related to the El Niño-Southern Oscillation (ENSO) phenomenon during summer. El Niño events during summer favour diatom growth due to an increase in upwelling of nitrate-rich water in Cook Strait and its subsequent advection into Beatrix Bay. During neutral or La Niña phases, dinoflagellate biomass tended to be high during summer due to a reduction in coastal upwelling.

Advection of phytoplankton into Beatrix Bay plays a major role in the spatial variation of phytoplankton across Beatrix Bay throughout the year. The main channel outside the bay generally has higher nitrate concentrations year round, and is well-

mixed due to high tidal velocities. The western side of Beatrix Bay has higher hydrodynamic exchange with the main channel than the eastern side of the bay. During summer, when nitrate is limiting to phytoplankton, biomass is higher in the main channel along with nutrient concentrations. Advection of this high biomass water into western Beatrix Bay results in a phytoplankton biomass that is generally higher there than in eastern Beatrix Bay. This community is comprised of a greater percentage of diatoms than the eastern side of the bay. During winter, phytoplankton in the well-mixed Pelorus channel are likely to be severely light-limited, and consequently biomass is lower outside Beatrix Bay. Advection of this low biomass water into western Beatrix Bay dilutes the phytoplankton concentration there, and biomass is generally lower in western Beatrix Bay than eastern Beatrix Bay at this time of the year.

Grazing by ciliates can potentially have a large effect on the biomass of small phytoplankton size classes. During one of the sampling trips, ciliate biomass was exceptionally high and significantly reduced phytoplankton biomass, despite growth conditions being ideal for phytoplankton.

Despite the Beatrix Bay phytoplankton community being comprised of dozens of phytoplankton taxa at any one time, community composition is generally predictable dominated by a few chain-forming diatom taxa, such as *Chaetoceros* sp., *Pseudonitzschia* sp., and *Skeletonema* sp., most of the time. These taxa can rapidly grow in response to nutrient input. Dinoflagellates only attain a high biomass during summer, when low nutrient concentrations and a thermally stratified water column creates conditions favourable to them. However, during El Niño events in summer, increased nutrient access due to coastal upwelling favours diatoms over dinoflagellates.

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CHAPTER 1
General Introduction

1.1 INTRODUCTION

This thesis is an investigation into phytoplankton community dynamics in a coastal ecosystem. Coastal ecosystems, which include tidal rivers, estuaries, embayments, lagoons, and coastal river plumes, are distinctly different from the open ocean (Moore 1958; Lalli and Parsons 1993; Cloern 1996). They are influenced by exchange with the open ocean and inputs from the land. Input from the land is often via rivers or tributaries carrying runoff from catchments. Riverine inputs include fresh water, nutrients, and sediment, and create a unique habitat characterised by a spatial gradient in environmental conditions along the river-ocean continuum (Cloern 1996).

The most important factors that influence phytoplankton growth and population dynamics are the supply of inorganic nutrients (D'Elia et al. 1986; Andersson et al. 1996; Carlsson and Graneli 1999), the availability of light (Cloern et al. 1985; Diehl 2002), and grazing pressure (Kivi et al. 1993; Archer et al. 2000). Stratification of the water column is important in determining the supply of nutrients and light to phytoplankton, as well as affecting the rate of sinking losses of cells (Diehl 2002). Stratification can be thermally-driven by vertical changes in water column temperature, and/or salinity-driven by vertical changes in salinity. The pycnocline, the region of rapid density change in a stratified water column, acts as a barrier to vertical water circulation (Lalli and Parsons 1993). Phytoplankton trapped in the surface layer of a stratified water column are exposed to higher mean irradiance than the average irradiance throughout the water column. However, replenishment of nutrients into the surface layer from bottom waters is limited in stratified systems, and the upper layer can rapidly become exhausted of nutrients due to phytoplankton uptake (Margalef 1978). Phytoplankton are denser than their surrounding medium, and non-motile diatoms tend to sink out of stratified water columns that lack the vertical turbulence required by diatoms to maintain their vertical position (Margalef 1978; Mann 1993). The motile dinoflagellates are able to maintain their position under weakly turbulent stratified conditions. In some conditions, maximum phytoplankton biomass occurs within the pycnocline, where access to nutrients from below and light from above are sufficient for phytoplankton growth (Cullen 1982). In a well-mixed water column, nutrient supply from bottom waters is enhanced but

average irradiance throughout the water column in which phytoplankton are mixed is reduced.

A fundamental and widespread feature of phytoplankton dynamics in marine and estuarine waters is sporadic rapid population increase, events often referred to as "blooms" (Paerl 1988; Legendre 1990). Phytoplankton blooms are prominent features of coastal ecosystems (Cloern 1996), as there are several characteristics of these ecosystems that make them ideal systems for phytoplankton blooms to occur. Such characteristics include:

1. Many coastal ecosystems are rich in nutrients due to the elevated nutrient inputs from adjacent land (Parsons et al. 1984; Mann and Lazier 1991). Anthropogenic nutrient enhancement is common in many coastal ecosystems through agricultural and municipal sources (Justic et al. 1995).
2. Riverine inputs of freshwater are a source of low-density water that promotes stratification of the water column, isolating the phytoplankton in an upper layer of relatively high mean irradiance. The semi-enclosed nature and reduced fetch of many coastal ecosystems can dampen the influence of wind as a source of kinetic energy to mix the water column, further aiding stratification.
3. Populations of deep-water zooplankton grazers that enter oceanic surface waters at night may be absent from shallow coastal ecosystems, depending on the depth of the ecosystem (Moore 1958). However, in some coastal ecosystems, grazers such as bivalves that live on the bottom may replace zooplankton grazers in exerting top-down control (e.g., San Francisco Bay (Alpine and Cloern 1992)).

The potential for phytoplankton production in coastal ecosystems can be much higher than in other oceanic regions, and population fluctuations in these ecosystems are highly amplified (Cloern 1996).

Phytoplankton biomass varies at all the spatial and temporal scales that have been measured (Cloern 1996; Smayda 1998). The potential for phytoplankton to bloom is set by the responses of phytoplankton to physical variability (bottom-up forcing) (Paerl 1988; Legendre 1990), and the scale of bloom variability is often determined by the scale of physical variability (Legendre and Demers 1984). These can be semidiurnal, daily and weekly tidal fluctuations, seasonal fluctuations in solar irradiance, and seasonal, episodic, and interannual

fluctuations in water column stratification, river flow, wind stress, and nutrient levels. Population changes in phytoplankton are not only due to *in situ* processes within a water parcel, but also horizontal transports that displace or mix water parcels and their phytoplankton. Intense top-down grazing pressure may prevent a phytoplankton bloom occurring despite ideal bottom-up resource conditions (Legendre 1990; Verity and Smetacek 1996). The focus of this study was on the influence of bottom-up processes in structuring the phytoplankton community in a coastal ecosystem.

1.2 IMPORTANCE OF PHYTOPLANKTON SPECIES

While the general conditions that drive phytoplankton blooms in coastal ecosystems are well researched and understood, an understanding of the community composition of these blooms and the processes that select for blooms of certain species are currently poorly understood (Cloern 1996). Phytoplankton are extremely diverse with an estimated 4000 to 5000 marine species described (Sournia et al. 1991; Tett and Barton 1995). The most abundant classes of marine phytoplankton are the diatoms and dinoflagellates (Lalli and Parsons 1993). Diatoms are non-motile cells capable of rapid cell division (Furnas 1990). Some taxa can form colonies or chains comprised of hundreds of cells. Dinoflagellates have two flagella, and most are motile and capable of vertical migration in the water column (Cullen 1982). This allows them to exploit sunlight near the surface during the day and nutrients below the pycnocline at night (Margalef 1978).

Many studies on phytoplankton, for convenience, focus on measurements of the total biomass as an index of phytoplankton dynamics. However, this offers a limited perspective that ignores species population dynamics. Individual phytoplankton species are at the base of the marine food web and are hence important for fisheries. They fuel the production of higher trophic levels that supply around 120 million tonnes of food to the world each year (FAO website 2004). Different species of phytoplankton can differ widely in their effect on the higher trophic levels that consume them (Verity and Smetacek 1996). For example, dinoflagellates are considered three to five times more nutritionally valuable per unit volume than diatoms (Chan 1980; Hitchcock 1982). Estuarine and coastal species of copepods will preferentially select dinoflagellates and microzooplankton over diatoms as food items (Kleppel et al. 1991). Blooms of many phytoplankton species can be toxic to higher trophic levels. Well-known examples of this are red tide blooms. For example, a red tide of the

dinoflagellate *Ptychodiscus brevis* can cause reproductive failure and mortality in bivalves (Summerson and Peterson 1990).

Blooms of phytoplankton can also act as agents of geochemical change that are highly species-dependent. Diatoms and silicoflagellates, for which silica is an important structural component, influence silica cycling in the ocean by transforming dissolved silicate into particulate skeletal material (Treguer et al. 1995). Rapid production of the climatically active trace gas dimethylsulfide in coastal waters is related to blooms of only particular phytoplankton species such as *Gyrodinium aureolum* (Turner et al. 1988) and *Emiliania huxleyi* (Wolfe et al. 1994).

Phytoplankton consists of a community of species that behave differently and have different effects on the environment and on higher trophic levels. Understanding the key processes that structure phytoplankton at the species level, and stimulate individual species to bloom, is key to an understanding of population fluctuations of higher trophic levels and the impact of phytoplankton on the environment.

1.3 PHYTOPLANKTON MEASUREMENT

The microscopic cell size, abundance of cells, and diversity of species makes phytoplankton communities difficult to assay. Numerous methods of phytoplankton measurement have been employed in studies, but no one method is accepted as being superior under all circumstances and for all purposes (Olivieri 1985). The most commonly used method to estimate phytoplankton biomass is the measurement of chlorophyll *a* concentration (Cullen 1982). Chlorophyll *a* concentration is the best chemical indicator of phytoplankton biomass, as only photosynthetic organisms contain the pigment (Cullen 1982). The measurement of chlorophyll *a* is also rapid and easy. However, the consistency of the method in estimating biomass is not assured because chlorophyll *a*: biomass ratios may vary depending on nutrient levels (Olivieri 1985; Falkowski and Raven 1997), light levels (Falkowski and Kiefer 1985), and phytoplankton species composition (Burns 1977; Falkowski and Raven 1997). Chlorophyll *a* measurement also reveals nothing about phytoplankton community composition.

In this study microscopy was the primary method used to assay the phytoplankton community. This was supplemented with chlorophyll *a* measurement. Microscopy provides excellent detail of community composition. The major drawback of this method is that it is very time consuming. Although artefacts may be incurred through the counting of sub-samples (Lund et al. 1958) and the shrinking of cells during preservation (Olivieri 1985), microscopy is accepted as the best method of measurement when investigating phytoplankton community composition and species numbers (Sournia 1978).

1.4 EXPERIMENTING IN THE OCEAN

Experimenting in the ocean presents considerable difficulty. Whereas terrestrial systems are characterised by a physical environment that does not move, in aquatic systems the physical environment and its associated organisms are in constant motion. The horizontal and vertical motion of aquatic systems makes it difficult to revisit the same populations of organisms over time. Large-scale *in situ* experiments, while providing increased confidence that the same organisms are being revisited, are logistically challenging, expensive, and rarely done. An example of successful large-scale *in situ* experiments are mesoscale additions of the micronutrient iron in the Southern Ocean, which have demonstrated iron limitation of phytoplankton growth (e.g., Cooper et al. 1996; Boyd et al. 2000). However, most studies on phytoplankton dynamics are limited to highly structured sampling of phytoplankton in conjunction with hydrographic measurements (e.g., salinity, temperature, nutrients), and experiments within enclosures that are designed to have inferential application to a larger scale.

Enclosures are experimental tools that allow population changes from known physical and/or chemical alterations to be measured while maintaining some of the natural environmental conditions (Menzel 1980). In this way potentially confounding effects of multiple influencing variables can be controlled. Enclosing the water undoubtedly introduces artefacts. Vertical and horizontal mixing, which can alter nutrient levels, light levels, grazer levels, and phytoplankton community composition, is eliminated or reduced within enclosures (Davis 1982). Grazers within an enclosure may have an impact that is disproportionate to their impact in an unenclosed environment (Downing et al. 1999). The succession of phytoplankton taxa observed within an enclosure is typically an accelerated version of succession observed in the marine environment (Davis 1982). While artefacts will always be

introduced when water is enclosed, enclosure experiments allow specific questions to be addressed that descriptive sampling techniques or pure laboratory techniques cannot address (Menzel 1980).

Enclosure experiments have been widely used in phytoplankton ecology, mainly to test the effects of nutrient enrichment (e.g., D'Elia et al. 1986; Hein and Riemann 1995; Carlsson and Graneli 1999), light limitation (e.g., Gallegos and Platt 1982; Huisman 1999; Huisman et al. 1999), grazing (e.g., Edgar and Green 1994; Prins et al. 1995a, b; Levine et al. 1999; Ogilvie et al. 2003) and to observe species succession (e.g., Brockmann et al. 1977; Sommer 1991). In this thesis structured sampling was used in conjunction with enclosure experiments to investigate processes driving phytoplankton community dynamics in a coastal ecosystem.

1.5 STUDY AREA

This study used Beatrix Bay as an example of a coastal ecosystem. Beatrix Bay is located within Pelorus Sound, a steep-sided, drowned river valley system (Cotton 1952) in the northeast of New Zealand's South Island (Fig. 1.1). The sound is approximately 50 km long with an area of 290 km^2 (Heath 1976a). Pelorus Sound is made up of a central channel with several large side arms and a continuous band of embayments on each side. The average depth of the sound is 25 m. At the head of the sound, depth does not exceed 10 m, while in the main channel the depth may exceed 70 m (Fig. 1.1). Pelorus Sound is commercially important and produces the majority of New Zealand's mussel aquaculture.

The Pelorus River, located at the head of Pelorus Sound (Fig. 1.1), is the major source of fresh water into Pelorus Sound with a mean fresh water inflow of approximately 43 m^3 s^{-1} (Heath 1974). Water column stratification, nutrient levels, and salinity vary from the inner sound to the outer sound. Salinity is low at the head of the sound and the water column is usually stratified, while towards the outer sound salinity is similar to the open ocean and there tends to be little density stratification (Heath 1976b, 1982; Gibbs et al. 1991). Thermal stratification of the water column also periodically occurs throughout the sound, particularly in embayments during summer when solar irradiance is high (Sutton and Hadfield 1997; Ross et al. 1998a; Gibbs et al. 2002). Circulation in the main channel is typically estuarine, with low salinity water travelling outwards from the head of the sound along the surface, and high salinity oceanic water flowing inwards along the bottom (Gibbs et al. 1991). The residence

time of water in Pelorus Sound is estimated to be about 20 days (Heath 1974; Heath 1976a). Pelorus Sound receives nitrogen from riverine inflows, advection of oceanic water from Cook Strait, and from sediment nutrient release (Bradford et al. 1986, 1987; MacKenzie et al. 1986; Gibbs et al. 1991, 1992, 2002; Gibbs 1993; Proctor and Hadfield 1996; Ross et al. 1998a).

Beatrix Bay (41°1'S, 174°01'E), a flat-bottomed bay approximately 35 m deep with a diameter of about 4.5 km, is part of a large side arm off the main Pelorus channel also consisting of Crail Bay and Clova Bay (Fig. 1.1, Fig. 1.2). Located about 30km from the Pelorus River mouth and 20km from the mouth of Cook Strait, Beatrix Bay is near the middle of the river-ocean continuum of Pelorus Sound. Beatrix Bay has been the focus of a major study on mussel aquaculture sustainability (Cole and Grange 1996; Hadfield and Sutton 1996; Proctor and Hadfield 1996, 1998; Gibbs and Vant 1997; Sutton and Hadfield 1997; Ross et al. 1998a, b; Gall et al. 2000; Ogilvie et al. 2000, 2003; Gibbs et al. 2002; Safi and Gibbs 2003). The existing knowledge on nutrient cycling and hydrodynamics in the bay make it an ideal place for a further study of phytoplankton community dynamics.

The Beatrix Bay water column is stratified most of the time by thermal and/or salinity stratification (Proctor and Hadfield 1996, 1998; Sutton and Hadfield 1997), and this has important implications for the nutrient dynamics of the embayment. Because the upper and lower water columns are frequently decoupled due to stratification, nitrogen regeneration from the sediments is seldom available to the upper water column within Beatrix Bay (Gibbs et al. 2002). Instead, the majority of dissolved inorganic nitrogen in the upper water column is advected into Beatrix Bay via the main Pelorus channel (Gibbs et al. 1992, 2002; Dupra 2000). The major source of this dissolved inorganic nitrogen is oceanic water from Cook Strait entering the Pelorus channel (Gibbs et al. 1992). Table 1.1 shows the relative magnitudes of dissolved inorganic nitrogen sources calculated by Gibbs et al. (1992). Cook Strait advection and sediment nutrient release contribute an estimated 30.4 t d^{-1} and 23.4 t d^{-1} of dissolved inorganic nitrogen to Pelorus Sound, compared with 0.9 t d^{-1} from river inflows (Table 1.1). This is in contrast to many other coastal ecosystems, whose main source of dissolved inorganic nitrogen is typically from riverine inputs and nitrogen regeneration from the sediment (Malone et al. 1988; Justic et al. 1995; Cloern 1996).

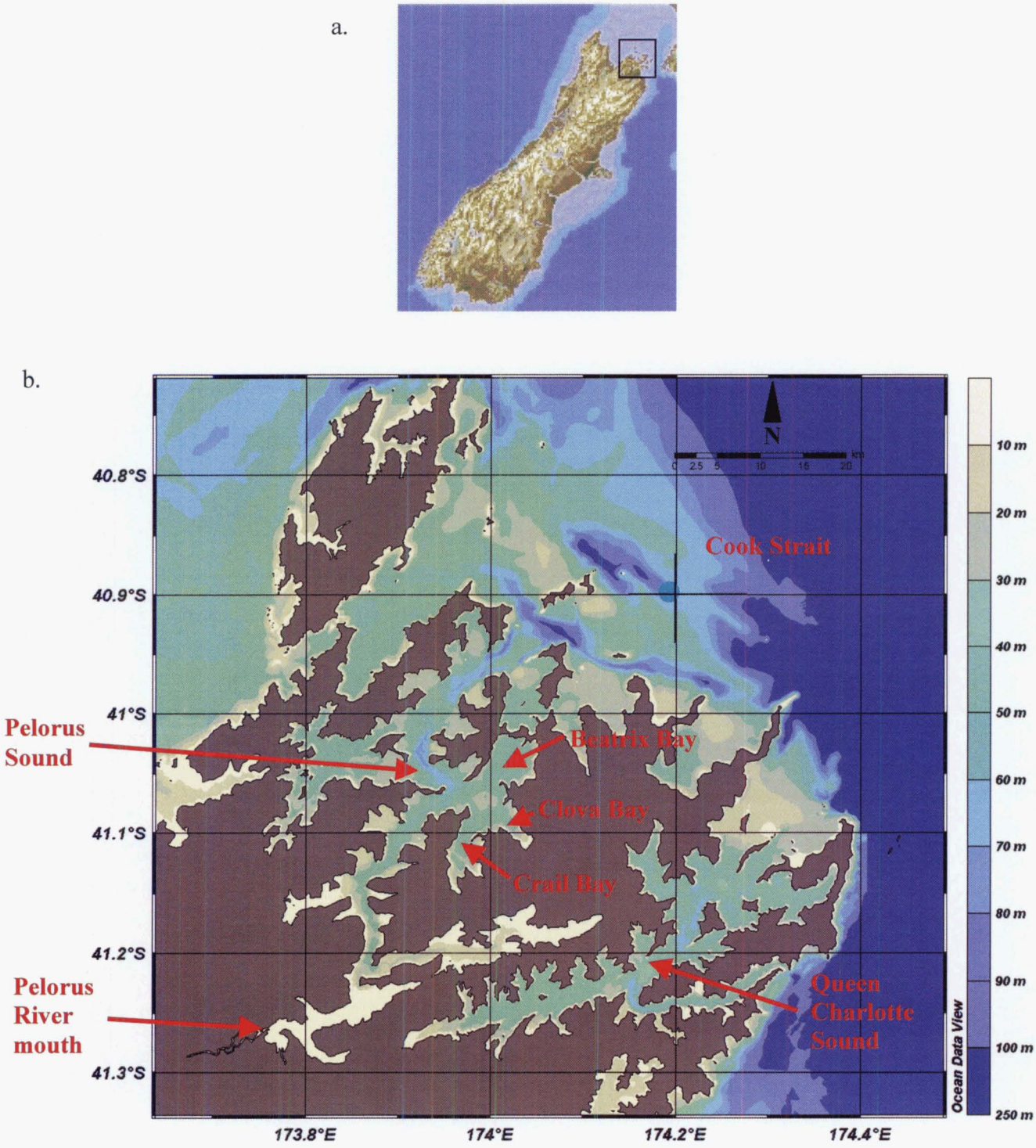


Figure 1.1. a) Map of the South Island of New Zealand showing the location of the Marlborough Sounds. b) Map of the Marlborough Sounds showing Pelorus Sound, Queen Charlotte Sound, Cook Strait, Beatrix Bay, Crail Bay, Clova Bay, and the Pelorus River mouth. Depth contours are indicated by colour bar on the right. Maps derived from Ocean Data View 5.5.

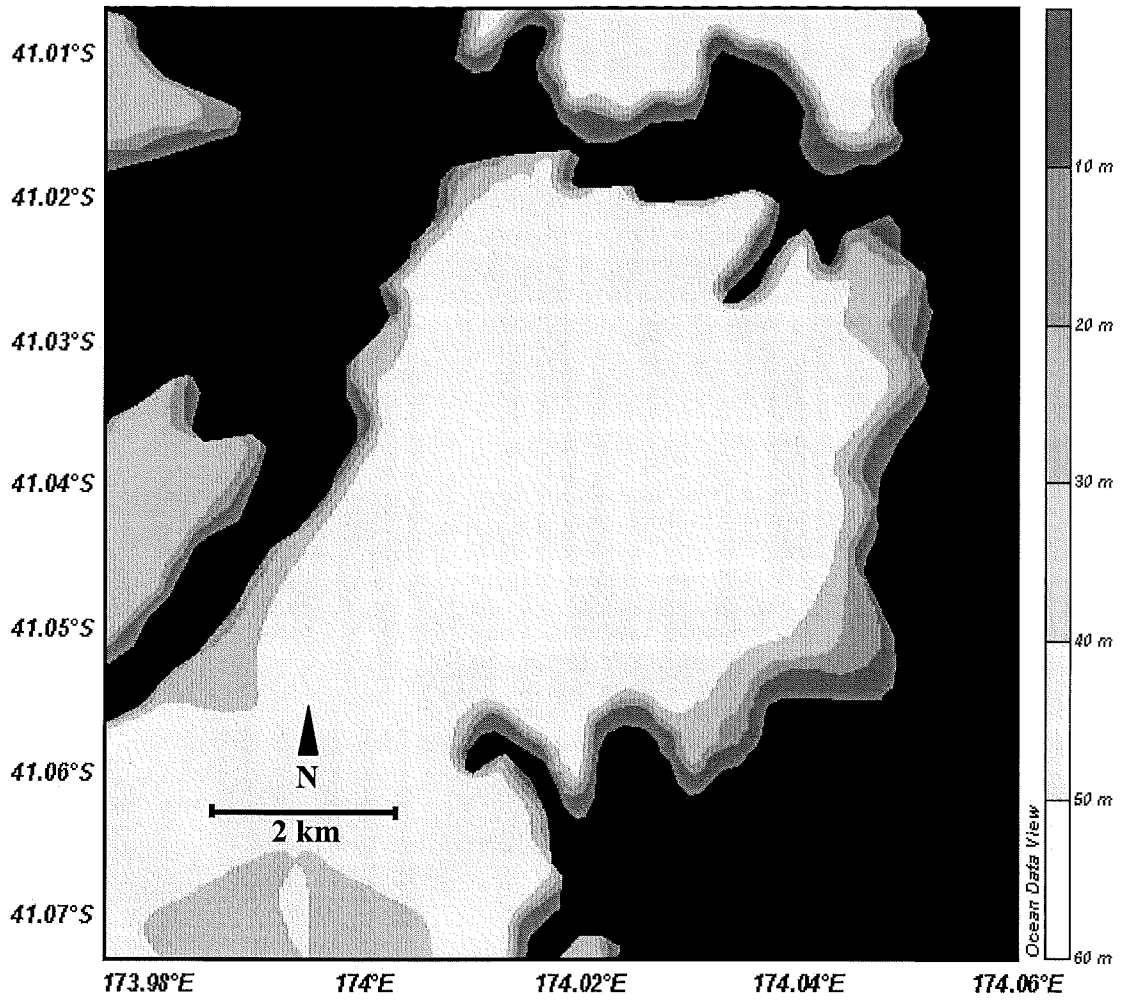


Figure 1.2. Map of Beatrix Bay with depth contours indicated by scale bar on the right. Map derived from Ocean Data View 5.5.

Table 1.1. Estimated relative magnitude of dissolved inorganic nitrogen sources to Pelorus Sound, reproduced from Gibbs et al. (1992).

Source	DIN Input (t d^{-1})
Cook Strait advection[a]	30.4
Sediment release[b]	23.4
River inflows[c]	0.9
Mussel N excretion[d]	0.7
Sewage[e]	0.0068

[a]Calculation based on mean residence time of 20 days (Heath 1976)
[b]Sediment N release estimated from Kaspar et al. (1985)
[c]From Shearer (1989a)
[d]Calculation based on 280 mussel farms, using excretion data from James et al. (1987)
[e]From Shearer (1989b)

The residence time of water in Beatrix Bay is estimated to be between five and eight days (Hadfield and Sutton 1996; Proctor and Hadfield 1996). Circulation patterns within the bay indicate predominantly clockwise circulation, with the western side of the bay having a greater level of exchange with the outside channel than the eastern side (Proctor and Hadfield 1996, 1998; Sutton and Hadfield 1997). This is demonstrated by a tracer simulation of hydrodynamic exchange calculated by Proctor and Hadfield (1996) (Fig. 1.3). The simulation divides Pelorus Sound water into water originating within Beatrix Bay at zero tidal cycles (yellow) and water from outside the bay at zero tidal cycles (blue). The simulation shows water from the main Pelorus channel being advected into Beatrix Bay along the western side. After four tidal cycles, water in northeastern Beatrix Bay is still predominantly water that originated from within the bay. Because most of the nitrate within the Beatrix Bay upper water column is advected in from outside, the spatially varying rates of exchange with the outside channel raise the possibility that access to nutrients may vary across Beatrix Bay. This would have important implications for the spatial dynamics of the phytoplankton within the bay.

Phytoplankton production in Beatrix Bay tends to be nitrogen-limited during spring and summer (Gibbs and Vant 1997; Ogilvie et al. 2003), a time when nitrate concentration in the upper water column can be virtually negligible (Ross et al. 1998b). Phytoplankton production tends to be light-limited during winter (Gibbs and Vant 1997), a time when nitrate levels are high but solar irradiance level is low. The critical depth, above which total photosynthetic production in the water column is balanced by total respiratory losses (Lalli and Parsons 1993), ranges from an average of 60 m depth in summer to 10 m in winter (Gall et al. 2000).

1.6 COMMERCIAL IMPORTANCE OF PELORUS SOUND PHYTOPLANKTON

The phytoplankton community dynamics in Pelorus Sound are not only scientifically relevant, but are important from a commercial perspective. Cultivation of the indigenous green-lipped mussel *Perna canaliculus* Gmelin 1791 has grown to become New Zealand's major aquaculture industry (Gall et al. 2000). Pelorus Sound is part of the Marlborough Sounds, a region that produces around 80% of New Zealand's mussel aquaculture (James and Ross 1996). Pelorus Sound is the most heavily farmed of the two major sounds, cultivating over 70% of the regions mussels (Fox 2003).

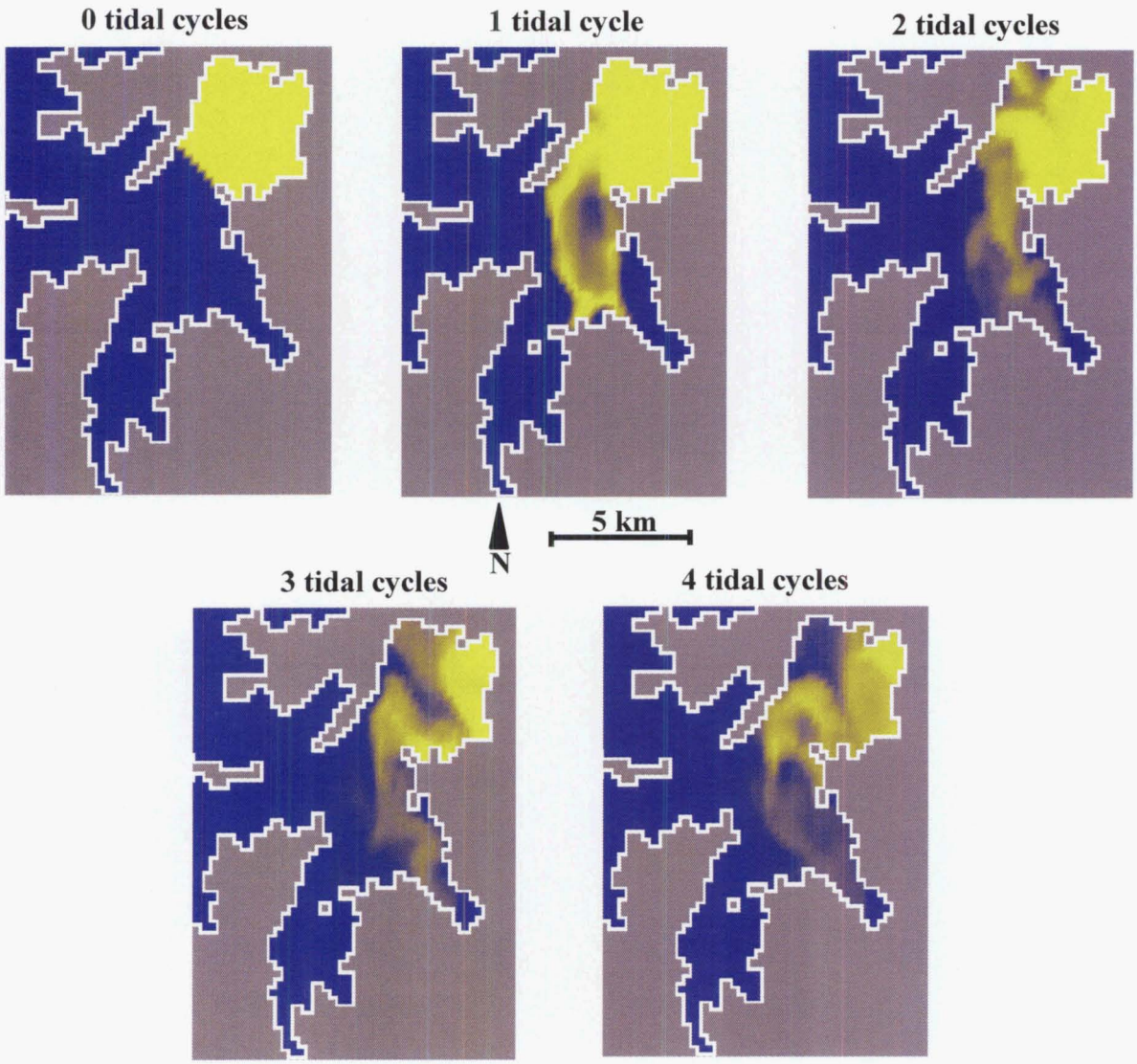


Figure 1.3. Tracer simulation of Proctor and Hadfield (1996) for Beatrix Bay showing hydrodynamic exchange of water inside and outside the bay over four tidal cycles. Yellow colour indicates water originating within Beatrix Bay after zero tidal cycles. Blue colour indicates water originating outside Beatrix Bay after zero tidal cycles. Water originating from outside Beatrix Bay can be seen travelling up the western side of the bay and mixing with water in western Beatrix Bay. After four tidal cycles, water in northeastern Beatrix Bay is still predominantly water that originated from within the bay. Wind stress is likely to have an influence on this hydrodynamic exchange.

Mussels are filter feeders and rely primarily on a natural supply of phytoplankton for growth (Smaal and van Stralen 1990; James and Ross 1996, 1997). There is considerable evidence that mussel growth can be limited by phytoplankton concentration and flux both in cultured (Meredyth-Young 1983; Waite 1989; Hickman et al. 1991) and natural populations (Wright et al. 1982; Wildish and Kristmanson 1984; Frechette and Bourget 1985 a,b; Frechette et al. 1989). Therefore the industry is heavily reliant on mussels receiving an adequate supply of phytoplankton.

It is not only phytoplankton quantity that is important. Food quality is also an important factor in bivalve nutrition (Beukema and Cadee 1991; Navarro et al. 1991; Hawkins et al. 1999). The quality of phytoplankton as food for mussels can differ widely among taxa (Prins et al. 1995a). Size range is also important because mussels feed less efficiently on phytoplankton that are outside their optimal size range of 2-200 μm (Ward et al. 1998; Safi and Gibbs 2003). Phytoplankton less than 2 μm in diameter have been found on occasion to comprise a large proportion of phytoplankton biomass at mussel farming sites in New Zealand (Safi and Gibbs 2003). Certain species of phytoplankton may also be toxic to mussels and/or humans (Prins et al. 1998). An example of this was an outbreak of shellfish toxicity due to a bloom of the dinoflagellate *Gymnodinium breve* in January 1993 (Clarke 1993; O'Hara 1993). The danger to human health led to the temporary closure of commercial and recreational shellfisheries throughout New Zealand.

Mussel farming in New Zealand began in the late 1960's as a viable alternative to the collapsed dredge fishery (Hickman 1989). By 2001 nationwide production had reached approximately 70 000 tonnes of mussels from 2500 ha of farms (Lupi 2001). There are resource consent applications for new mussel farms covering an additional 8000 ha (MacKay 2000). Included are proposals for 'mega-farms', the largest covering 1600 ha. In the Marlborough Sounds, there is the potential for one-third of the coastal seabed to be allocated to marine farms (MacKay 2000). Future farms may no longer be restricted to a band within 200 m of the shoreline but may be located in the middle of bays.

Beatrix Bay has a high density of mussel farms, with around forty farms around the coastline within 200 m of the shore (Fig. 1.4). The influence of the intensive mussel farming within Beatrix Bay on phytoplankton is not fully understood. Cultured mussels have been found to

have a localised effect on phytoplankton biomass within farms in Beatrix Bay (Ogilvie et al. 2000). During autumn and winter, phytoplankton levels were significantly reduced due to mussel grazing within mussel farms. During summer, when ambient nutrient levels are low, phytoplankton levels were significantly higher within mussel farms. It was thought that this localised increase was due to the fact that mussels excrete dissolved inorganic nitrogen, which may supplement low ambient concentrations (Ogilvie et al. 2000).

With a potential increase in mussel biomass in Pelorus Sound, long-term sustainability of the industry has become a major issue. Because phytoplankton quantity and quality are important, an understanding of community dynamics of phytoplankton is vital to the industry.

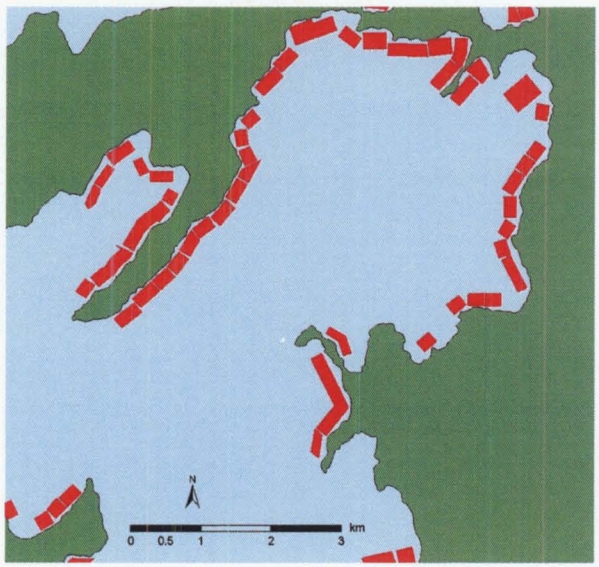


Figure 1.4. Map of Beatrix Bay in Pelorus Sound. Current mussel farm sites are shown in red.

1.7 OBSERVATIONAL BASIS FOR STUDY

I had access to a nine-year data set of phytoplankton species biomass and nutrient concentration in Beatrix Bay for this study. The data set was available courtesy of the NIWA weekly monitoring program and formed the observational basis of this thesis from which my hypotheses were generated. The data set consisted of single weekly 15 m integrated samples from the upper mixed layer, collected as a measure of phytoplankton biomass and nutrient levels in the photic zone. Vertical variability down the water column was not considered here.

Phytoplankton in Beatrix Bay between 1995 and 2002 was characterised by a series of fluctuations in biomass (Fig. 1.5). Three scales of variability are evident from which questions arose:

- Short-term sporadic variability- for example, peaks in diatom biomass that followed in rapid succession within a season such as in mid-1995 and mid-1998
    - What are the factors that cause these peaks in biomass?
    - What is the taxonomic composition of these blooms? Are the same taxa regularly blooming or are these peaks in biomass comprised of different taxa?

- Seasonal variability- for example, diatom biomass generally peaked in winter/spring and dinoflagellate biomass generally peaked during summer. The timing and magnitude of these seasonal blooms changed from year to year, a phenomenon that applies generally to coastal ecosystems (Cloern 1996).
    - What are the factors that drive this seasonal succession of taxa?

- Interannual variability- this is particularly evident when examining dinoflagellate biomass. During the summers of 1994-95, 1996-97, 1997-98, and 2000-01, the summer blooms of dinoflagellates were suppressed. The summers of 1996-97 and 1997-98 were characterised by higher than normal diatom biomass.
    - What drives this interannual variability in phytoplankton dynamics in Beatrix Bay?

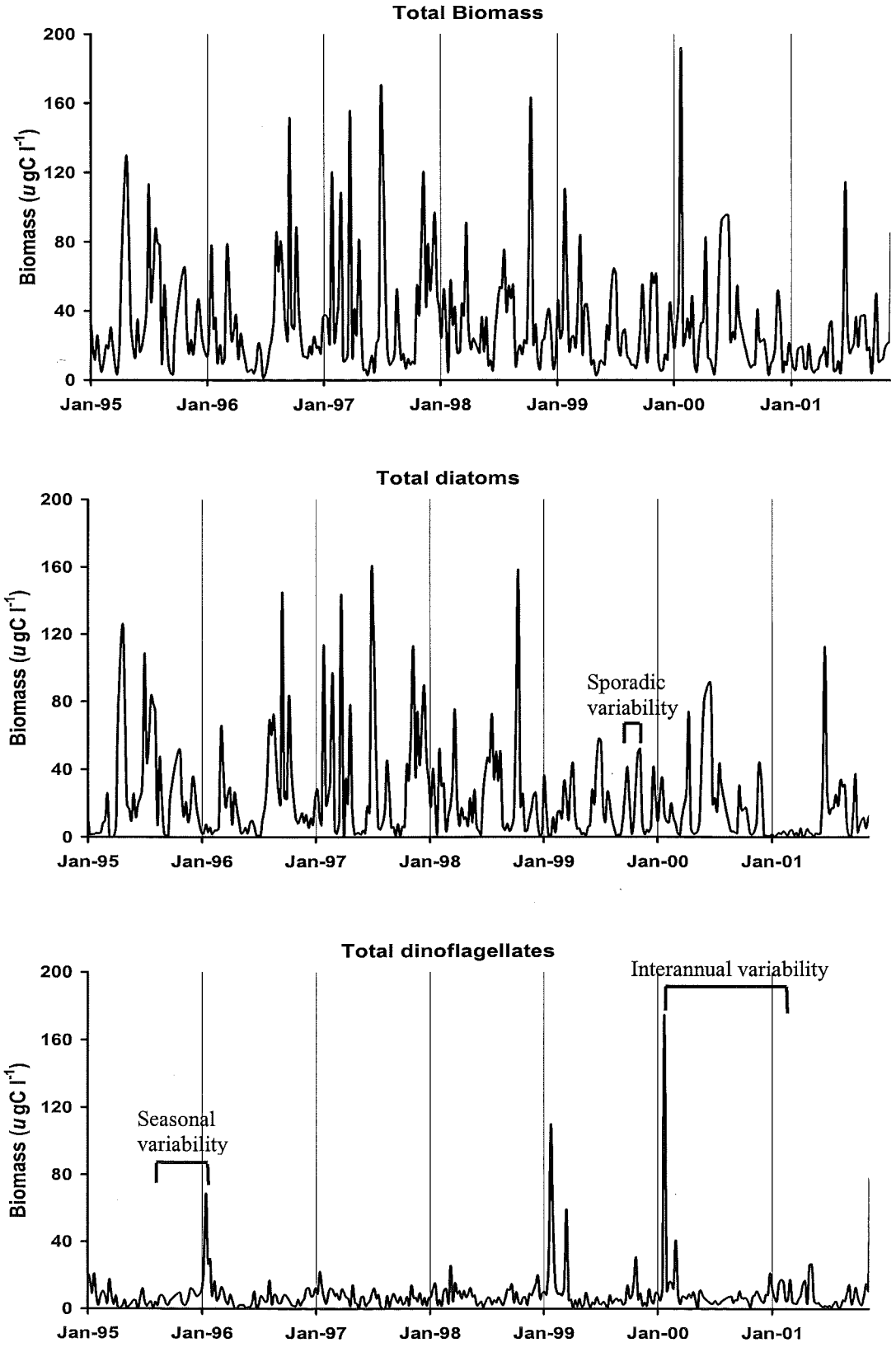


Figure 1.5. Total biomass, diatom biomass, and dinoflagellate biomass in Beatrix Bay between 1995 and 2002. Data represent single weekly depth-integrated samples. Data courtesy of NIWA monitoring program.

In conjunction with these temporal observations, questions arose about the role of spatial processes in driving phytoplankton variability.

- Are peaks in biomass caused by *in situ* processes within the bay, or does horizontal transport of cells into the bay play a major role?
- Are phytoplankton dynamics in Beatrix Bay likely to be influenced by localised processes or larger scale, Sounds-wide processes?

1.8 OBJECTIVES

This thesis is divided into three sub-objectives that comprise the three results chapters (Chapters 3-5). The sub-objectives all contribute to the overall objective of determining the processes that structure the phytoplankton community in Beatrix Bay. An outline of the main questions examined in each chapter is given below.

Chapter 3. Factors driving episodic and seasonal phytoplankton dynamics

This chapter investigates how nutrients, light, ciliate grazers and water column structure control phytoplankton community dynamics both seasonally and sporadically. Enclosure experiments, that manipulated nutrient and light levels, were used to investigate key factors controlling seasonal and short-term variability of the phytoplankton community. *In situ* sampling was used to relate experimental conditions to ambient conditions.

The hypotheses investigated were:

- That nitrate levels are limiting to phytoplankton for part of the year
- That the response to nitrate enrichment varies between phytoplankton taxa, and differences in response are based on taxonomic and morphological characteristics of the taxa
- That the most important factor structuring the phytoplankton community is the response of different taxa to nitrate levels
- That light levels are limiting to phytoplankton for part of the year
- That the response to light reduction varies between phytoplankton taxa, and differences in response are based on taxonomic and morphological characteristics of the taxa
- That the most important factor structuring the phytoplankton community is the response of different taxa to light levels

- That phytoplankton blooms can be suppressed by intense microzooplankton grazing even when growth conditions are favourable

Chapter 4. Spatial variability of Beatrix Bay phytoplankton

Are taxa blooms in Beatrix Bay caused by advection of cells or generated within the bay? Because of the circulation patterns of Beatrix Bay, the western side of the bay has greater hydrodynamic exchange with outside channel, which is the main nitrate source to the upper water column. Drogues were deployed to follow the water currents in an attempt to confirm these circulation patterns. This chapter investigates spatial variability in nutrient levels and phytoplankton at sites across Beatrix Bay. The aim is to determine the extent to which advection processes as opposed to within-bay processes are important in driving phytoplankton community composition in Beatrix Bay.

The hypotheses tested were:

- That exchange with the outside channel differs across Beatrix Bay
- That nitrate levels are consistently higher in western Beatrix Bay than eastern Beatrix Bay
- That phytoplankton in eastern Beatrix Bay are more nitrate-limited than phytoplankton in western Beatrix Bay
- That phytoplankton biomass and community composition differs across Beatrix Bay, and is associated with the varying hydrodynamic exchange and nutrient levels across the bay

Chapter 5. Factors driving long-term phytoplankton dynamics: Influence of El Niño-Southern Oscillation

In this chapter it is proposed that interannual phytoplankton variability in Beatrix Bay is associated with large-scale climatic influences, particularly the El Niño-Southern Oscillation (ENSO) phenomenon. Two ENSO-driven mechanisms that could potentially influence phytoplankton in Beatrix Bay were investigated. They are:

- That ENSO affects Beatrix Bay phytoplankton through climate-driven changes in upwelling in Cook Strait, thereby altering nitrate import into Beatrix Bay at interannual time scales

- That ENSO affects Beatrix Bay phytoplankton through climate-driven rainfall anomalies altering Pelorus River flow, thereby affecting the degree of salinity stratification in Beatrix Bay at interannual time scales

These hypotheses were investigated by examining the long-term relationships between

1. ENSO and (i) upwelling in Cook Strait and (ii) Pelorus River flow
2. Beatrix Bay phytoplankton and (i) upwelling in Cook Strait and (ii) Pelorus River flow

Chapter 6. General Discussion

The General Discussion ties the results of Chapters 3, 4, and 5 together to address the overall objective of understanding the phytoplankton community dynamics in Beatrix Bay.

CHAPTER 2
General Methods

2.1. INTRODUCTION

This chapter describes the general sampling, experimental, and data analysis techniques used in this thesis in the following format:

- NIWA weekly monitoring program
- Description of field sampling for physical and chemical conditions of the water column, and for phytoplankton biomass
- Description of sample analysis methods
- Description of microcosm experiments
- Data analyses

The methods that were specific to particular sections of the thesis are presented in each chapter separately.

2.2 NIWA WEEKLY MONITORING PROGRAM

A weekly monitoring program for the determination of nutrient concentration and phytoplankton taxa biomass in Beatrix Bay began in October 1994 and is still ongoing. An additional site outside Beatrix Bay (OB) was included in the monitoring program in January 1997. Samples were collected from three sites: western Beatrix Bay (WB), eastern Beatrix Bay (EB), and outside Beatrix Bay (OB) (Fig. 2.1). Sampling consisted of a single weekly sample from each site.

Integrated water samples for phytoplankton identification and nutrient analysis were collected from the top 15 m of the water column using a 20 mm diameter clear plastic hose. Vertical variability of phytoplankton and nutrients was not measured. Samples were analysed for phytoplankton species composition by the Cawthron Institute using the inverted microscope technique described by Utermöhl (1958). The cell counts were converted to cell biovolume by multiplying the cell counts by the mean individual cell volume of each group. The mean cell volumes are listed in Appendix 1. Cell carbon ($\mu gC\ l^{-1}$) was estimated by converting cell biovolume using regression equations for dinoflagellates, diatoms and small flagellates (see Safi and Gibbs 2003). Nutrient samples were filtered through Whatman 25 mm GF/F glass

microfibre filters, under reduced pressure (<20% reduction), in a positive-pressure clean-air laboratory. The water was analysed for nitrate-plus-nitrite N (hereafter referred to as nitrate NO_3-N) and total ammoniacal N (hereafter referred to as ammonium NH_4-N) on an AlpKem series 500 air-segmented continuous flow marine auto-analyser using the methods of Grasshoff et al. (1983). Detection limits in μM were: NO_3-N 0.04; NH_4-N 0.07.

2.3 FIELD SAMPLING

Field sampling for this study consisted of a series of five-day sampling trips done approximately bi-monthly over almost two years. The months during which sampling trips were undertaken are listed in Table 2.1. Sampling trips consisted of daily field sampling in conjunction with enclosure experiments. Field sampling was conducted at three sites in and around Beatrix Bay: West Beatrix, East Beatrix, and Outer Beatrix (Fig. 2.1). Triplicate water samples for the determination of chlorophyll *a* concentration, size-fractionated chlorophyll *a* concentration, nutrient (nitrate, ammonium, phosphate, and silicate) concentration, and phytoplankton taxa identification were taken daily at each site during the five-day duration of each sampling trip. Water column structure was also measured by a single daily APV cast at each site during sampling trips.

Table 2.1. Month and year in which field sampling trips were carried out.

Year	Month
2001	April
	August
	December
2002	January
	March
	May
	July
	September
	November
2003	February

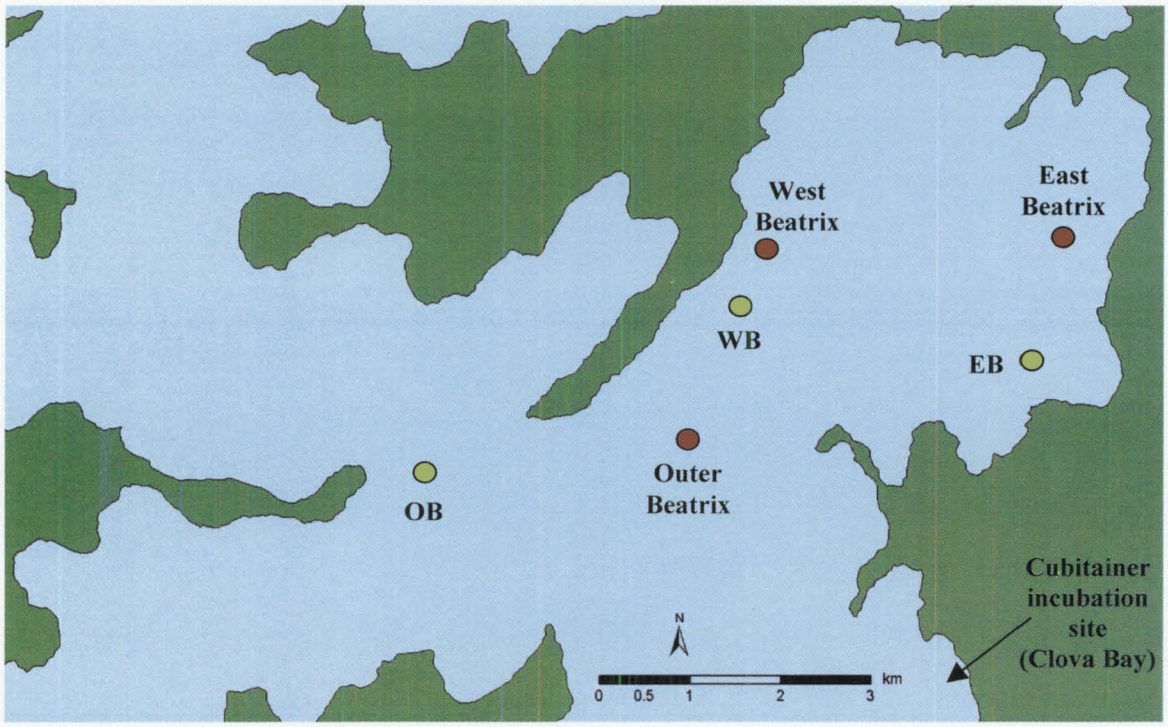


Figure 2.1. Map of Beatrix Bay showing study sites. NIWA weekly monitoring program sites are marked with yellow dots. Field sampling sites are marked by red dots. The cubitainer incubation site near the entrance to Clova Bay is also shown.

2.3.1 Water Column Structure

Water column structure was measured using an Ocean Sensors Model OS200 APV profiler fitted with a WETStar miniature fluorometer (Fig. 2.2). A calibration check using standards of known salinity and temperature was carried out prior to each sampling trip. Fluorescence was measured at a wavelength of 470 nm. The fluorometer was calibrated within two weeks of field sampling by comparing instrument readings with laboratory-derived fluorescence of known standards. The instrument was programmed to measure conductivity, temperature, and chlorophyll *a* fluorescence 99 times per second as it was slowly lowered and raised through the water column.

Relationship between chlorophyll a and tidal cycle

To investigate whether chlorophyll *a* varied with tidal cycle an Ocean Sensors Model OS200 APV profiler fitted with a WETStar miniature fluorometer (Fig. 2.2) was attached to the outer longline of a mussel farm in eastern Beatrix Bay. The APV was fixed at 5 m depth for 48 h

(approximately four tidal cycles). The instrument was re-programmed to measure chlorophyll *a* fluorescence 99 times every five minutes. There was no relationship between chlorophyll *a* and tidal cycle during this time period (Fig. 2.3). Ogilivie (2000) also found no relationship between chlorophyll *a* and tidal cycle in a previous study in Beatrix Bay. This allowed subsequent phytoplankton sampling to be done regardless of the tidal cycle.

2.3.2 Integrated Sampler

Water samples for determination of chlorophyll *a* concentration, size-fractionated chlorophyll *a* concentration, nutrient (nitrate, ammonium, phosphate, and silicate) concentration, and phytoplankton taxa identification were collected using an integrated sampler (Venrick 1978). The sampler consisted of a transparent tube that was 25 mm in diameter, 10 m long and weighted at the bottom (Fig. 2.4). This was designed to sample the upper 10 m of the water column, above the pycnocline. When the water column is stratified in Beatrix Bay the pycnocline is typically below 10 m deep (Hadfield and Sutton 1996; Proctor and Hadfield 1996). Vertical variability of phytoplankton and nutrient concentration was not measured.

The tube was lowered until it was fully extended in the water column. A rubber stopper was then inserted into the upper end of the tube and the lower end was pulled up using a rope attached at the bottom. The integrated sample was emptied into a clean, 20 l plastic bucket through the bottom end by taking the rubber bung out of the top end. The sample was then gently poured into a clean, labelled 1 l bottle and placed in a dark insulated container during transport back to the laboratory. Samples for the determination of chlorophyll *a* and size-fractionated chlorophyll *a* concentration were filtered within three hours of collection and frozen prior to analysis. Samples of filtrate were collected in 125 ml acid-washed polyethylene bottles for the determination of nutrient concentration and frozen prior to analysis.

2.4 SAMPLE ANALYSES

2.4.1 Chlorophyll *a* analyses

Chlorophyll *a* samples were filtered onto Whatman 25 mm GF/F glass microfibre filters under reduced pressure and frozen. For analysis chlorophyll *a* filters were ground to homogeneity, and the chlorophyll *a* extracted into 10 ml of 90% acetone. Chlorophyll *a*

Figure 2.2. Ocean Sensors Model OS200 APV profiler fitted with WETStar miniature fluorometer.

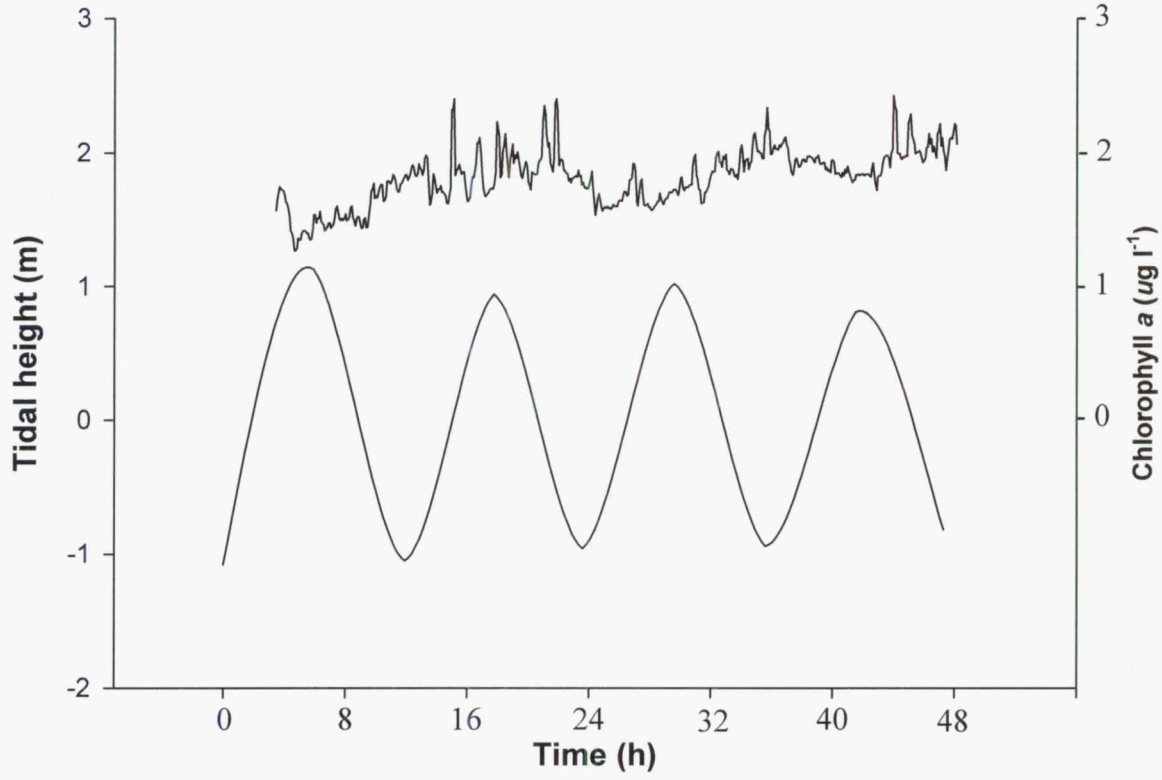


Figure 2.3. Chlorophyll *a* concentration (top line) over approximately four tidal cycles at a mussel farm in eastern Beatrix Bay. Data collected by WETStar miniature fluorometer moored at 5 m depth measuring chlorophyll *a* fluorescence 99 times every five minutes. Bottom line indicates tidal height. Tidal data courtesy of the NIWA Tide Forecaster.

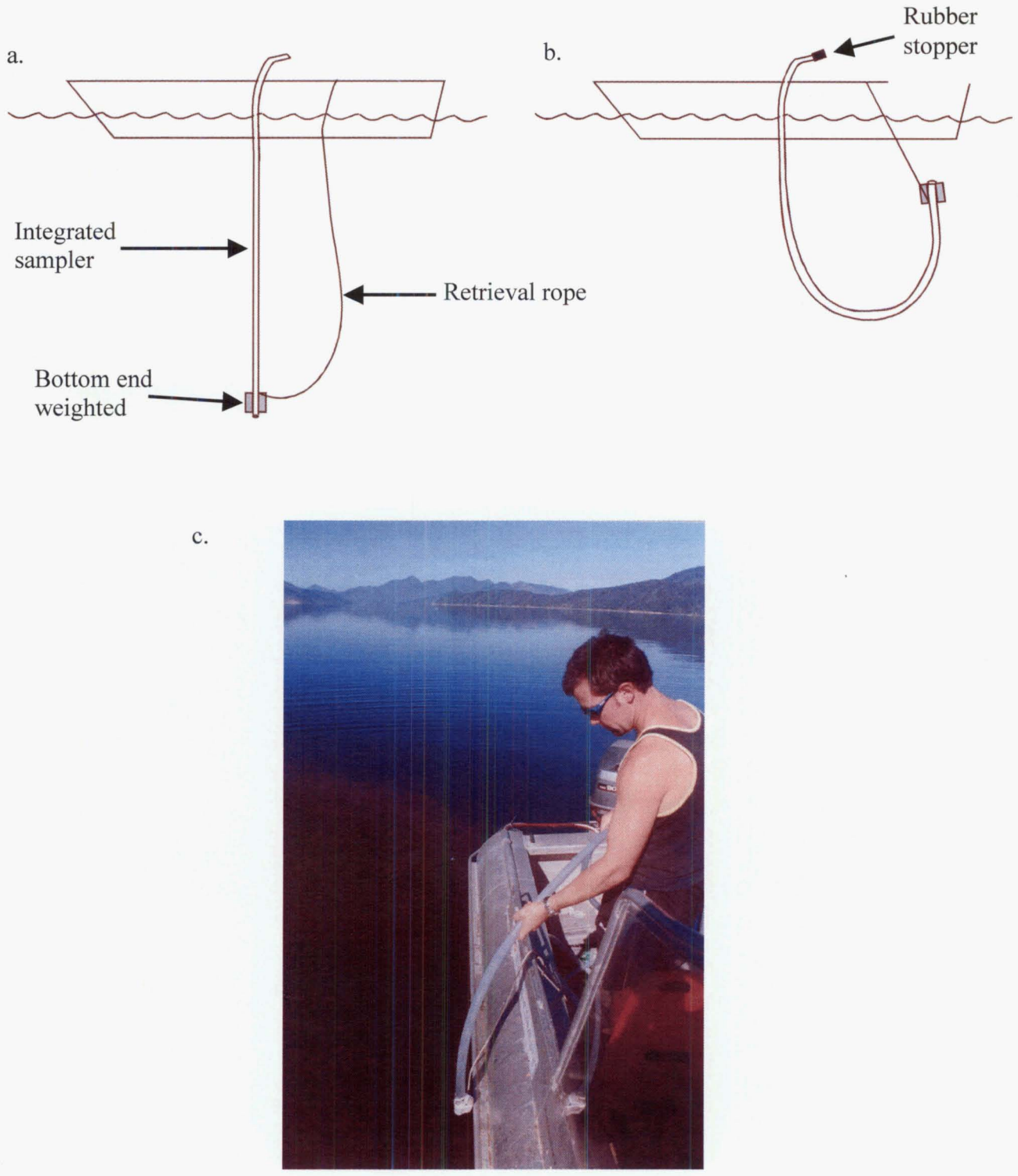


Figure 2.4. a) Diagram of integrated sampler deployment. b) Diagram of integrated sampler retrieval, showing rubber stopper closing the top end and the sampler being pulled up using the rope attached to the bottom end. c) Photograph of integrated sampler deployment in Beatrix Bay.

concentrations (with phaeophyton correction following acidification in 0.1 M HCl), were determined with a Perkin-Elmer fluorometer (Strickland and Parsons 1968). Size-fractionated chlorophyll *a* samples were filtered onto Osmonics 47 mm polycarbonate filters (0.2, 2, and 20 μm) under reduced pressure. This divided the phytoplankton into three size classes:

- Microphytoplankton >20 μm diameter
- Nanophytoplankton 2-20 μm diameter
- Picophytoplankton <2 μm diameter

Size-fractionated chlorophyll *a* concentrations were determined following 24 hr soaking in 5 ml of 90% acetone.

2.4.2 Phytoplankton taxa cell counts

Phytoplankton taxa samples were preserved using acidified Lugol's iodine (APHA 1992), as recommended for inshore marine areas by Throndsen (1978). A 250 ml sub-sample was allowed to settle for 24 hours. The sub-sample was then decanted to 25 ml and placed in an Utermöhl chamber. This was settled for a further 24 hours so that all the phytoplankton cells in the 250 ml sub-sample were settled onto the slide of the Utermöhl chamber. Samples were observed at 200x magnification under a Wild M40-82720 inverted microscope, as described by Utermöhl (1958). For each sample, 20 fields of view were counted, as in Ogilvie (2000). The objective was to count at least 400 cells in each sample, giving a precision of ± 10% from the mean (Lund et al. 1958). If 20 fields of view yielded less than 400 cells, additional fields of view were examined until 400 cells had been counted.

Seventy-seven taxonomic groupings, to coincide with those used in the NIWA weekly monitoring program, were counted. These taxa represented the species level, genus level, or a higher level of taxonomic grouping. The taxa used are listed in Appendix 1. Identification of each taxonomic group was done with the guidance of Lebour (1978), Dodge (1980), Dodge (1985), Sournia (1986), Chretiennot-Dinet (1990), Round et al. (1990), Larson and Moestrup (1992) and Hallegraeff et al. (1995). Cell counts were also converted to biovolume by multiplying cell counts by the mean individual cell volume of each group. Mean individual cell volumes were taken from a database made available to me by Karl Safi (National Institute of Water and Atmospheric Research, Hamilton). The mean cell volumes are listed in Appendix 1.

2.4.3 Nutrient analyses

The filtrate, following filtration through Whatman 25 mm GF/F glass microfibre filters, was collected in 125 ml plastic acid-washed bottles and frozen prior to analysis. Nitrate plus nitrite N (hereafter referred to as nitrate), total ammoniacal N (hereafter referred to as ammonium), dissolved reactive phosphorus (hereafter referred to as phosphate) and dissolved silica (hereafter referred to as silicate) concentrations were analysed using a Technicon continuous flow air segmented autoanalyser. Nitrate was estimated as the sum of nitrate plus nitrite by the cadmium reduction method (Grasshof 1970), ammonium by the indophenol blue method (Mantoura and Woodward 1983), phosphate by the molybdenum blue method (Downes 1978), and silicate by the method of Perstorp (1993).

2.5 ENCLOSURE EXPERIMENTS

To test the effects of nutrient concentration and light on phytoplankton community composition and biomass, experimental manipulations were undertaken using 12 litre polyethylene cubitainers (collapsible microcosms) (Fig 2.5). When filled the cubitainers became spheroid in shape with no sharp edges. The size of the enclosure is an important aspect in the design of aquatic enclosure experiments. As enclosure size increases, wall effects decrease. However, large size also increases the structural heterogeneity of the enclosed water and decreases the chance of duplicating systems (Gamble and Davies 1982). Hence, a compromise has to be reached regarding enclosure size. Enclosures for investigating nutrient limitation in phytoplankton typically range in size from 1 litre to 1 000 litres (Hecky and Kilham 1988). Twelve litre cubitainers were chosen for this study because:

(i) this size is adequate to investigate the short-term responses of phytoplankton taxa to nutrient enrichment and light reduction;

(ii) the small enclosure size allowed sufficient reproducibility so that it was manageable to carry out experiments with the desired number of treatments and replicates.

2.5.1 Treatments

The treatments were arranged in a full-factorial design. The treatments were:

- added-nitrate (+ nitrate/ambient light)
- added-nitrate and shaded (+ nitrate/- light)
- control (no manipulations) (ambient nitrate/ambient light)

- shaded (ambient nitrate/- light)

Nitrate Addition

At the start of each experiment a 10 ml nutrient spike solution in deionised water was added to each added-nitrate cubitainer. This was intended to raise the nitrate concentration in these cubitainers by at least 5 μM.

Light Reduction

The cubitainers were attached in random order to a rope strung out horizontally at 5 m depth from the backbone of a mussel farm in Clova Bay (Fig. 2.1, Fig. 2.6). Light attenuation was measured at one-metre depth intervals in the water column on four separate days during August 2001 using a LI-COR LI-192SA underwater quantum radiation sensor connected to a LI-COR LI-1000 data logger. Average light attenuation of the water column as a percentage

Figure 2.5. An example of a shaded (right) and an unshaded (left) cubitainer. Temperature loggers can be seen attached to the handles. Cubitainer dimensions are 23 cm^3.

of surface irradiance was calculated and is shown in Figure 2.7. The light attenuation of the cubitainer walls, measured with a LI-COR PAR hand-held radiation wand, was found to average 9.6%. When hung at 5 m depth in the water column, light levels within unshaded cubitainers simulated *in situ* irradiance at 5-6 m depth (Fig. 2.7). Shaded cubitainers were wrapped in black shadecloth that reduced light further to approximately 30% of ambient level. Light levels within shaded cubitainers simulated *in situ* irradiance at approximately 11 m depth (Fig. 2.7).

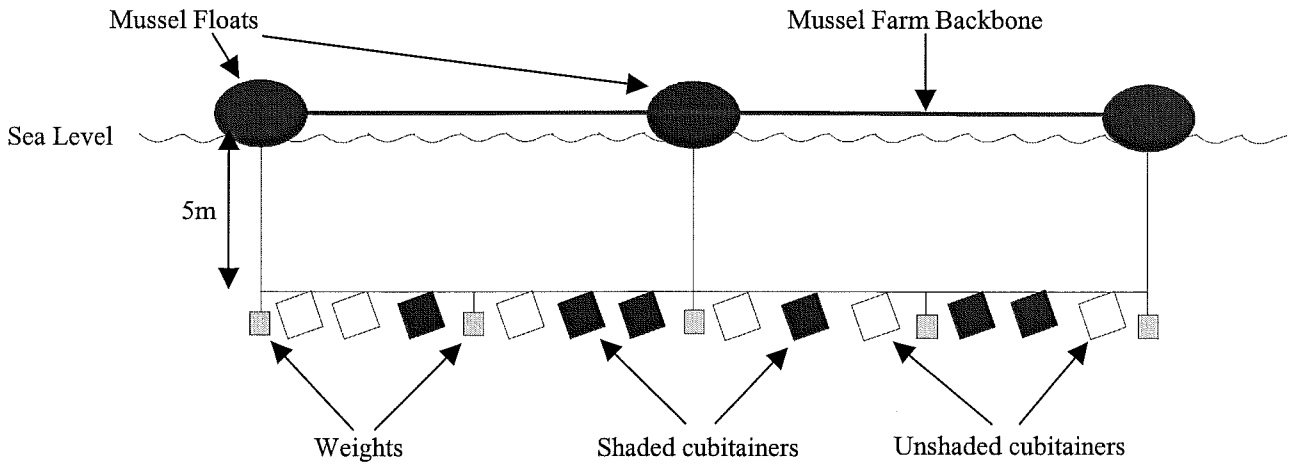


Figure 2.6. Cubitainer set-up during incubation. Cubitainers were attached to a rope strung horizontally at 5 m depth. The horizontal rope was attached to mussel floats along a mussel farm backbone at three points. The rope was weighted down by five 2 kg diving weights.

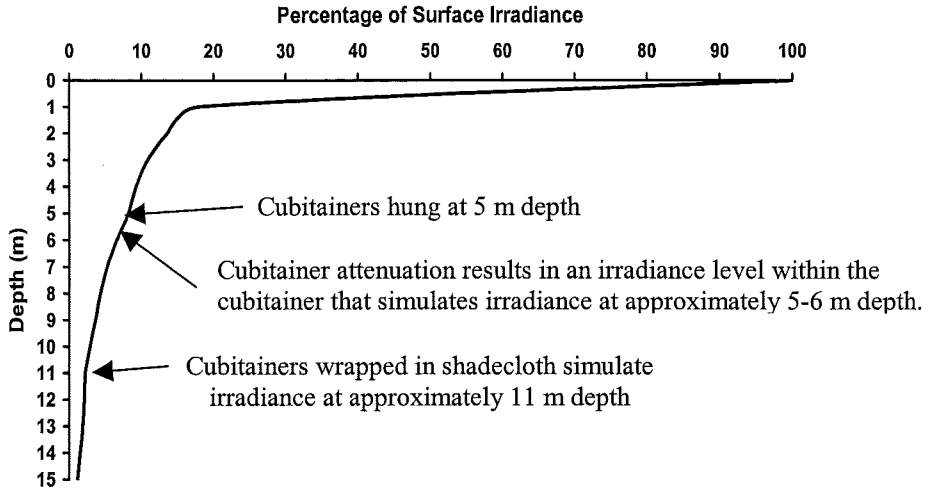


Figure 2.7. Average irradiance at one-metre depth intervals as a percentage of surface irradiance at the cubitainer incubation site in Clova Bay (Fig. 2.1). Average calculated from four profiles measured on different days during August 2001. Experimental cubitainers were hung at 5 m depth in the water column. Attenuation of cubitainer wall (9.6%) means that unshaded cubitainers simulated irradiance level at 5-6m depth and shaded (70% light reduction) cubitainers simulated irradiance levels at 11 m depth.

2.5.2 Cubitainer Filling

Water from several integrated tube samples was poured into a clean bucket and mixed. Water was sieved through a 250 μm mesh to remove macrozooplankton grazers, a common procedure in such experiments (Downing et al. 1999). Large grazers may have a very significant impact in a small enclosure that is disproportionate to their impact in an unenclosed environment, and may mask the phytoplankton responses that are the focus of the experiment. Microzooplankton (heterotrophic organisms <250 μm) were still present. They cannot be removed by sieving as they are similar in size to phytoplankton (Downing et al. 1999).

The cubitainers were partially filled in stages from a single bucket with all cubitainers sequentially filled a third at a time. This three-step filling was done in an attempt to improve initial homogeneity between cubitainers. The order of filling cubitainers was random. An air bubble was left in the cubitainers to aid mixing by wave action. The cubitainers were inverted to mix the water once daily, and immediately prior to sampling.

2.5.3 Incubation and sampling

The cubitainers were incubated *in situ* at 5 m depth (Fig. 2.6). *In situ* incubation allowed the cubitainers to be kept at the ambient water temperature, preventing temperature-dependent effects that may affect simulated surface incubations (Lohrenz et al. 1992). Six randomly selected cubitainers had Onset Stowaway TidbiT® waterproof temperature loggers (range – 5°C to 37°C) attached (Fig. 2.5) to record water temperature every two minutes throughout the experiments. This was done to ensure that phytoplankton response during the experiments could not be attributed to a large fluctuation in temperature. The cubitainers were incubated *in situ* for 4 days. This time interval was chosen to give an adequate response time of phytoplankton to the treatments, while minimising artefacts that exert greater effects through time such as microzooplankton grazing and the limitation of trace nutrients (Downing et al. 1999). In their review of marine nutrient-enrichment experiments, Downing et al. (1999) claimed that experiments lasting 1 day or less are too short to measure limitation, while experiments lasting more than 7 days are too long.

Each cubitainer was sampled both at the beginning and the end of the experiment by pouring water directly from the cubitainer into a clean, labelled 1 l bottle. Initial samples were taken immediately after any manipulations were administered (e.g. nitrate addition) to set initial conditions. The bottle was immediately placed in a dark insulated container for transport back to the laboratory. The cubitainer was then squeezed to maintain a small air bubble. Samples were taken for determination of chlorophyll *a* concentration, nutrient (nitrate, ammonium, phosphate, and silicate) concentration, and phytoplankton taxa identification.

2.5.4 Mesocosm Experiment

A mesocosm experiment was undertaken in March 2002 in conjunction with equivalent cubitainer experiments to investigate how closely results within cubitainers reflected what occurred on a larger scale. The mesocosms had a volume of 25 000 l. The large-scale experiment had to be aborted early due to storm damage. However, preliminary results displayed in Appendix 2 indicated that after one day, nutrient-enhanced production that occurred within the cubitainers mirrored that which occurred in a larger-scale system exposed to a similar increase in nutrients. Logistic difficulties and cost prevented a further attempt at a mesocosm experiment.

2.6 DATA ANALYSES

Data manipulation and collation was done using Microsoft Excel (2000). Phytoplankton taxa data were transferred to a Microsoft Access (2000) relational database for management due to the large amount of data. Counts of taxa were standardised to number of cells ml^{-1} and biovolume ml^{-1} using the volume of water sampled and the number of fields of view examined. Statsoft Statistica 6.0 was used for statistical analysis. Graphical presentation was done using Microsoft Excel, SigmaPlot (2001), or CorelDraw 9.0.

The designs of most of the sampling and experiments lent themselves to multifactorial analysis of variance (ANOVA). Prior to analysis, raw data were tested for the ANOVA assumptions of normality (normal probability plots of residuals) and homogeneity of variances (Cochran C test). Data that didn't meet these ANOVA assumptions were transformed using a log (x + 1), square root, or a 4^{th} root transformation. When data did not respond to transformation and the variances remained heterogeneous, ANOVA was continued with caution. ANOVA is robust and operates well even under considerable heterogeneity of variances provided sample sizes are approximately equal (Glass et al. 1972). Sample sizes were always equal when ANOVA was used in this thesis. Student-Newmann-Keuls tests were used for *post hoc* analysis. Spearman's rank order correlation was used to test for correlations between variables.

CHAPTER 3

Factors driving episodic and seasonal phytoplankton dynamics

3.1 INTRODUCTION

Work carried out for this chapter investigated the processes driving sporadic and seasonal phytoplankton variability in Beatrix Bay. Sporadic and seasonal phytoplankton blooms are fundamental features of coastal ecosystems (Cloern 1996). Blooms are formed in response to sporadic and seasonal variations in nutrient levels, irradiance levels, water column structure, grazing levels, and competition for resources between phytoplankton (Legendre and Demers 1984; Paerl 1988; Egge and Aksnes 1992). The processes that determine the species composition of these blooms are not well understood (Cloern 1996).

The success of individual phytoplankton groups are largely dictated by their taxonomic and morphological characteristics. Diatoms and dinoflagellates are the most abundant classes of marine phytoplankton (Lalli and Parsons 1993). These different life-forms are adapted to thrive in different environmental conditions (Margalef 1978). Most diatom taxa have rapid growth rates (Furnas 1990). They tend to thrive in late winter/spring when a shoaling of the water column traps phytoplankton cells in an upper layer where the mean irradiance level is high and nutrient concentration remains sufficient for growth (Moore 1958; Chang et al. 1992). Prolonged stratification of the water column leads to the upper layer becoming deficient in nutrients as primary producers use them up (Margalef 1978). Dinoflagellates typically prosper in these conditions as their motility enables them to exploit both the overlying euphotic zone and the underlying nutrient-rich waters (Margalef 1978; Cullen 1982; Mann 1993). The motility of dinoflagellates also enables them to maintain their position under the weakly turbulent conditions of a stratified water column (Margalef 1978; Mann 1993). Diatoms will generally sink out of a water column that is strongly stratified with low turbulence.

Size is an important characteristic in determining nutrient uptake efficiency and susceptibility to grazing. While smaller size offers increased nutrient uptake efficiency through a greater surface area to volume ratio, smaller size also increases susceptibility to grazing (Malone 1980). Many diatoms form chains that can be hundreds of cells in length. It is thought that

this evolved to prevent grazing without sacrificing nutrient uptake ability (Munk and Riley 1952).

The focus of this chapter was to investigate the major processes structuring sporadic and seasonal phytoplankton community dynamics in Beatrix Bay. The following hypotheses were tested:

- That nitrate levels are limiting to phytoplankton for part of the year
- That the response to nitrate enrichment varies between phytoplankton taxa, and differences in response are based on taxonomic and morphological characteristics of the taxa
- That the most important factor structuring the phytoplankton community is the response of different taxa to nitrate levels
- That light levels are limiting to phytoplankton for part of the year
- That the response to light reduction varies between phytoplankton taxa, and differences in response are based on taxonomic and morphological characteristics of the taxa
- That the most important factor structuring the phytoplankton community is the response of different taxa to light levels
- That phytoplankton blooms can be suppressed by intense microzooplankton grazing even when bottom-up conditions are favourable

These hypotheses were investigated using a combination of *in situ* sampling and enclosure experiments. Along with the NIWA weekly monitoring program data, sampling trips were conducted approximately bimonthly over almost two years. Measurements were made of the *in situ* water column structure, nutrient levels, and phytoplankton biomass and community composition. This provided temporal information on the phytoplankton community and the processes affecting it. In conjunction with these sampling trips, perturbation experiments were conducted, in which nutrient and light levels were manipulated in order to investigate the influence of these factors on seasonal phytoplankton community dynamics.

This study was primarily designed to investigate the influence of bottom-up processes on phytoplankton, and therefore the macrozooplankton (size range > 250 μm) was eliminated from all experimental enclosures by sieving. Microzooplankton (< 250 μm), comprised

mostly of ciliates, could not be eliminated as they are similar in size to phytoplankton. An experiment to estimate the impact of ciliate grazing rates on phytoplankton biomass was conducted to determine the extent to which phytoplankton blooms could be controlled by top-down microzooplankton grazing.

3.2 METHODS

3.2.1 Field study sampling times and site

In situ sampling and experimental study trips were carried out during the months listed in Table 2.1. The figures in this chapter that show these months all refer to this 2001-2003 time series. *In situ* samples and water for experimental manipulations were collected from the site West Beatrix in Beatrix Bay (41°02.503'S, 174°00.037'E) (Fig. 2.1).

3.2.2 *In Situ* Sampling

Water Column Structure

Daily APV casts for salinity and temperature at depth (see Chapter 2 for further information) were made at the sampling site to measure water column structure. Pelorus River flow data were collected from the NIWA standard stream gauge at Bryants (Fig. 3.1). The methodology for calculating flow is described in McKerchar (2002). Solar irradiance data were measured at Blenheim airport (Fig. 3.1), approximately 50 km from the study site (NIWA climate database).

Water Column Samples

In situ water column samples for determination of chlorophyll *a* concentration, size-fractionated chlorophyll *a* concentration, nutrient (nitrate, ammonium, phosphate, and silicate) concentration, and phytoplankton taxa identification were collected using an integrated tube sampler to cover the top 10 m of the mixed layer (see Chapter 2 for further description). Triplicate water column samples were taken daily during the five-day duration of each sampling trip. Methods for analysing chlorophyll *a*, phytoplankton taxa, and nutrients are outlined in Chapter 2.

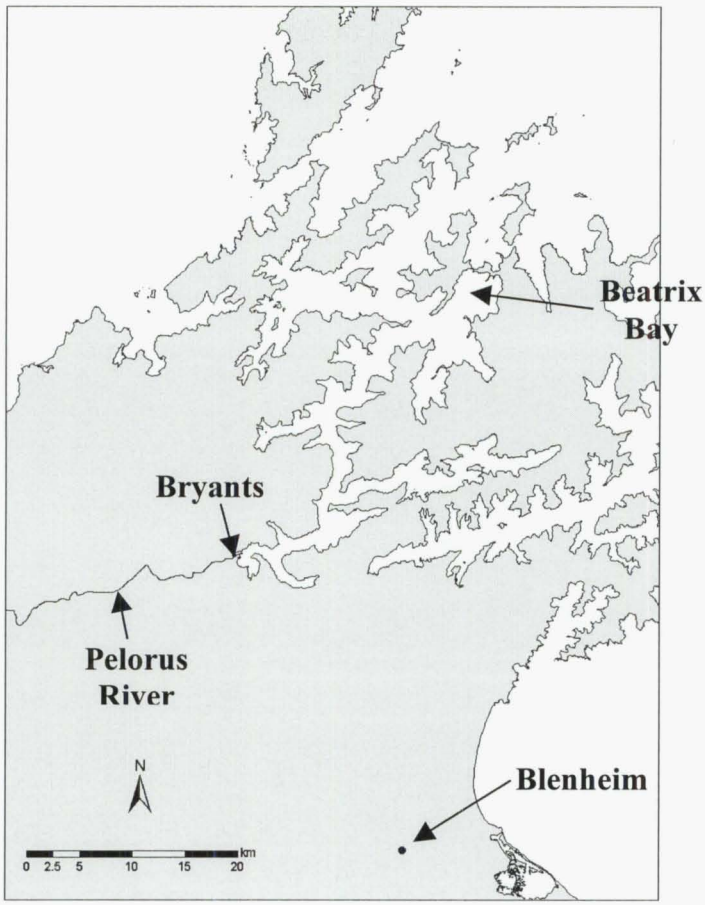


Figure 3.1. Map of Marlborough region showing Beatrix Bay, the Pelorus River, Bryants gauging station (where Pelorus River flow data were collected), and the town of Blenheim (where solar irradiance data were collected).

3.2.3 Weekly Time-Series Sampling

Weekly time-series data for phytoplankton biomass and nutrient concentration are courtesy of the NIWA weekly monitoring program in Beatrix Bay. Methods of sampling and analysis are outlined in Chapter 2. Samples were taken from the site WB (Fig. 2.1).

3.2.4 Nutrient and Shade Experiments

Enclosure experiments using 12 l cubitainers were carried out to test the effect of nitrate and light on phytoplankton community structure. These cubitainer experiments were conducted during each sampling trip to assess seasonal changes in these relationships. The protocol, the

cubitainers, and the design of the experiments are outlined in Chapter 2. The treatments were arranged in a full-factorial design. There were four treatments:

- added-nitrate (+ nitrate/ambient light)
- added-nitrate and shaded (+ nitrate/- light)
- control (no manipulations) (ambient nitrate/ambient light)
- shaded (ambient nitrate/- light)

There were three replicates of each treatment. Each cubitainer was sampled both at the beginning and the end of the experiment, which lasted for four days. Initial samples were taken immediately after any manipulations were administered (e.g. nitrate addition) to set initial conditions. Samples were taken for determination of chlorophyll *a*, nutrient (nitrate, ammonium, phosphate and silicate) concentration, and phytoplankton taxa identification. Each microcosm was inverted once daily to aid mixing. Microcosms were inverted immediately prior to sampling to ensure homogeneity in the experimental chamber. *In situ* temperature profiles from each experiment, collected by an attached Onset Stowaway TidbiT® temperature logger, are displayed in Appendix 3.

3.2.5 Grazing Experiment

An experiment to determine ciliate grazing rates on phytoplankton was conducted in February 2003. The experiment was based on the dilution method of Landry and Hassett (1982), which involves the sample water being diluted with filtered water from the test site. A range of dilutions was set up and the experiment was incubated for 1 day. Dilution of sample water means there is less likelihood of contact between predator and prey, and therefore lower grazing rates. The grazing rate is calculated from the measurement of grazing losses at several known dilutions.

Thirty litres of water from the upper 10 m of the mixed layer was collected by an integrated sampler, screened through a 250 μm mesh to remove macrozooplankton, and kept in a dark carboy while the dilution water was prepared. The dilution water consisted of 20 l of seawater filtered through a Gelman 0.2 μm gravity feed cartridge filter. The experiment was done in acid-washed 12 l cubitainers. All dispensing was carried out gently to avoid damaging cells. The < 250 μm screened water was diluted with 0.2 μm filtered water to concentrations of 10%, 40%, 70% and 100 % (undiluted). As nitrate limitation was anticipated to be a factor,

excess nitrate was added to cubitainers to increase the ambient concentration by 5 μM. This ensured that nutrient limitation of phytoplankton would not influence the results and that nitrate would be equally available to phytoplankton at all dilution levels (Landry and Hassett 1982). An additional set of undiluted cubitainers without added nitrate were run as controls (Landry and Hassett 1982; Landry 1993). There were three replicates of each treatment. The cubitainers were incubated at 5 m depth. Samples were taken for chlorophyll *a* and size-fractionated chlorophyll *a* (see Chapter 2 for size fractions) analysis at the beginning and end of the incubation.

The apparent growth rate (r) of phytoplankton was calculated, assuming exponential growth, by:

$$r = \ln (P_t/P_0)/t$$

where P_t is the concentration at t_1, P_0 is the initial concentration, and t is the time of incubation in days (Landry 1993). A plot of r versus X (proportion of undiluted water) should give a line with slope = -g (ciliate-specific grazing rate per day) and intercept = μ (phytoplankton-specific growth rate per day) (Landry and Hassett 1982). Linear response curves were only accepted when the relationship had an $R^2 > 50\%$ (Safi et al. 2002). Each linear response curve had a significant regression ($p<0.05$). An example of a linear response curve is shown in Figure 3.2.

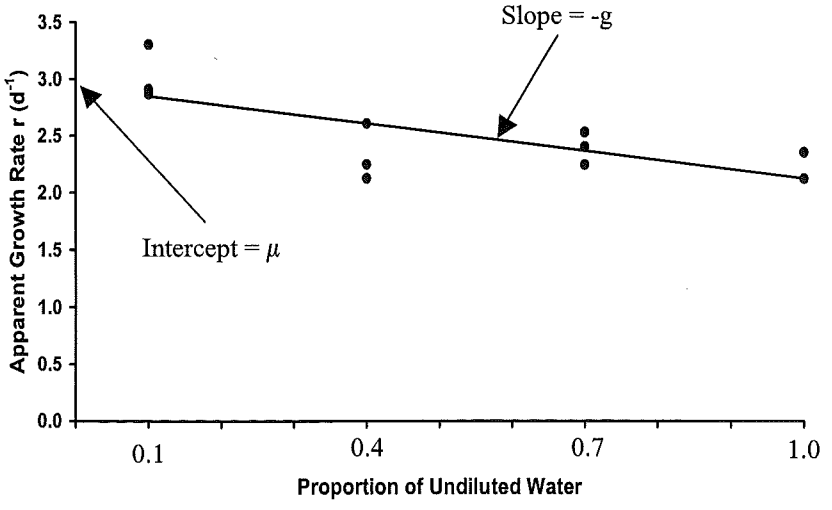


Figure 3.2. Example of a response curve from the microzooplankton grazing experiment. Data is from the microphytoplankton size class. Y intercept = μ (phytoplankton-specific growth rate per day). Slope of line of best fit = -g (ciliate-specific grazing rate per day). R^2 = 0.52.

3.2.6 Statistical Analyses

To compare experimental results between treatments and different times of the year three-way ANOVA was used with the independent factors being month, experimental nitrate level, and experimental light level. The dependent variable was the increase in phytoplankton during the experiment, calculated as final concentration/initial concentration. Phytoplankton taxa samples from the April 2001 experiment were not included in the analysis. More than half of these samples were completely lost due to leaking in the sample containers. Post-hoc comparison of response to the treatments during each month was made using Student-Newman-Keuls test.

3.3 RESULTS

3.3.1 Water column structure

The water column was strongly stratified during December, January, July and September (Fig. 3.3, Fig. 3.4). Further analysis of the water column profiles showed that during December there was very strong salinity stratification and strong thermal stratification, in January there was very strong thermal stratification and slight salinity stratification, and in July and September there was salinity stratification only (Table 3.1). The water column was weakly stratified during August, March and November (Fig. 3.3, Fig. 3.4). The water column was well mixed during April, May and February, as indicated by the small difference in density between 1m and 20m (Fig. 3.3). There was a negative density difference in February, meaning that denser water was overlying less dense water. This indicates that the water column was highly unstable at this time.

The sampling periods when strong salinity stratification occurred (December, January, July and September) came immediately after periods of extremely high Pelorus River flow (Fig. 3.5a). The Pelorus River is the major source of freshwater input into Pelorus Sound (Heath 1974). Although colder water is denser than warmer water, during the winter months (July, August, September) colder water was overlying warmer water (Table 3.1). This indicates that density differences were driven by differences in salinity at that time. During summer strong thermal stratification of the water column usually occurs as a result of convective heating, and the surface water layer was warmer (e.g. December, January). In spring and autumn the water column is in a transition between these two phases (A. Ross, pers. comm.). At this time, thermal stratification may be only moderate despite high levels of solar irradiance (e.g. November) (Table 3.1, Fig. 3.5b).

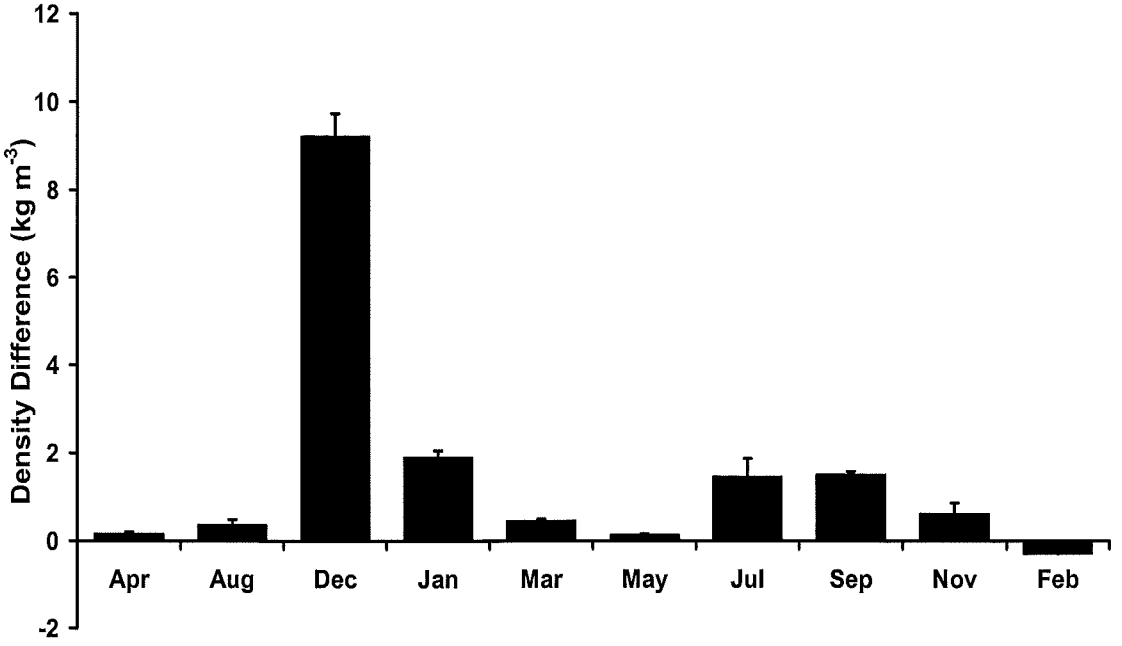


Figure 3.3. Change in water column density between 20 m and 1 m depth in Beatrix Bay. Measurements taken daily over a five-day period. Error bars are ± 1 SE.

Table 3.1. Differences in temperature and salinity between 1 m and 20 m deep. Temperature value is temperature at 1 m minus temperature at 20 m. Salinity value is salinity at 20 m minus salinity at 1 m. Standard deviations are in parentheses.

Month	Temperature (°C)	Salinity (ppt)
April	0.13 (0.08)	0.07 (0.06)
August	-0.18 (0.04)	0.64 (0.12)
December	2.01 (0.18)	10.13 (1.03)
January	2.29 (0.11)	1.80 (0.22)
March	0.73 (0.10)	0.35 (0.02)
May	0.03 (0.02)	0.19 (0.03)
July	-0.89 (0.51)	1.73 (0.77)
September	-0.06 (0.03)	1.93 (0.08)
November	0.58 (0.33)	-0.48 (0.72)
February	-0.47 (0)	-0.11 (0)

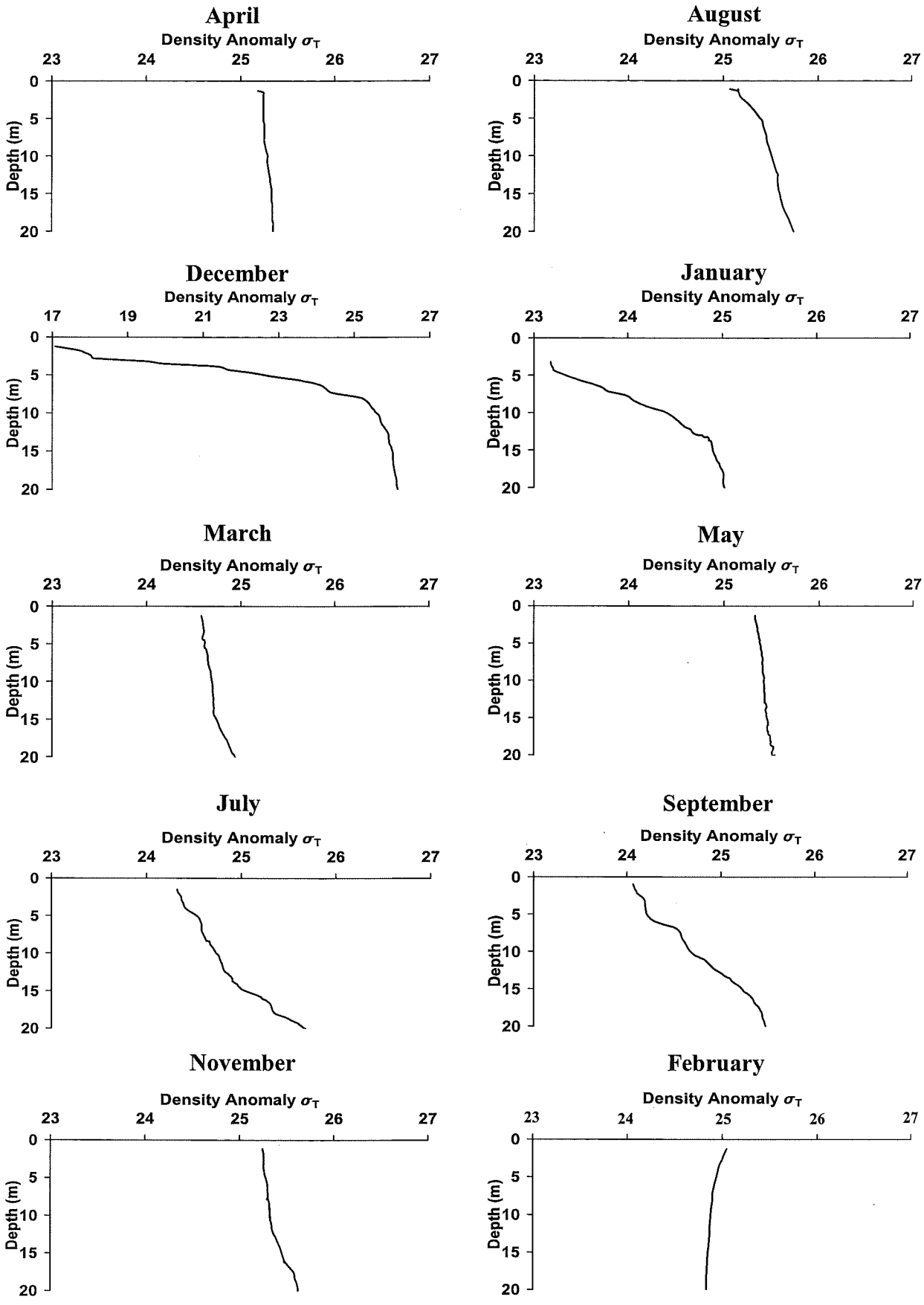


Figure 3.4. An example of a typical water column density profile in Beatrix Bay during study trips. Density anomaly (σ_T) is the difference from freshwater density. Density anomaly (σ_T) scale for December is broader than other months.

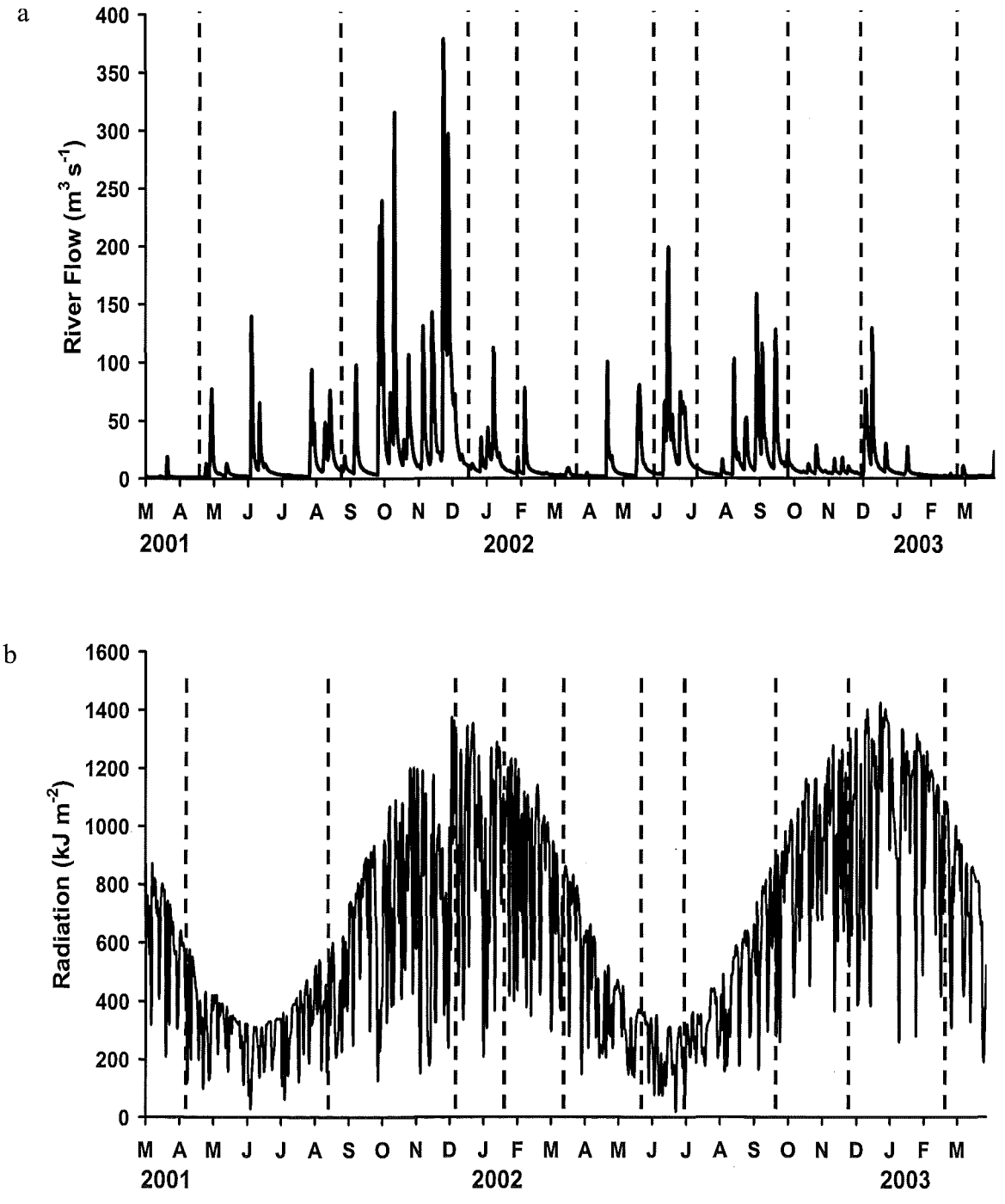


Figure 3.5. (a) Mean daily Pelorus River flow ($m^3 s^{-1}$) measured at Bryant's gauging station. (b) Mean daily solar irradiance ($kJ m^{-2}$) measured at Blenheim airport. Dotted lines indicate sampling periods. Data courtesy of the NIWA climate database.

3.3.2 Ambient nutrient concentrations

Nitrate, ammonium and phosphate concentrations varied seasonally, with low levels during the summer months and high levels during winter (Fig. 3.6a, b, c). Nitrate displayed the most extreme seasonal variability. During January 2002 ambient nitrate concentration was close to zero, averaging less than 0.002 μM (below the detection limit of ~ 0.04 μM). During July 2002 ambient nitrate concentration was the highest experienced during this study averaging 3.9 μM. Ammonium variation was not as pronounced as nitrate, with ammonium levels fluctuating from a low of 0.2 μM in December 2001 to a high of 0.4 μM in May 2002. Phosphate concentration ranged from 0.1 μM in December 2001 to 0.5 μM in May 2002. Silicate concentration did not show any seasonal pattern (Fig. 3.6d). Silicate levels were generally between 16.0 and 32.0 μM except for August 2001 (3.2 μM), December 2001 (64.5 μM) and November 2002 (8.6 μM).

3.3.3 Ambient phytoplankton levels

The two different methods of estimating phytoplankton biomass (chlorophyll *a* concentration and phytoplankton biovolume) showed similar seasonal patterns of low levels during summer months and high levels during winter (Fig. 3.7a, b). Picophytoplankton (<2μm) usually dominated phytoplankton biomass during the summer months (Fig. 3.7c), particularly in January 2002 and February 2003. Microphytoplankton (>20μm) usually dominated the phytoplankton biomass during the winter months, particularly May and July 2002. Diatom biovolume was greater than dinoflagellate biovolume during all months (Fig 3.7d). However, diatom dominance over dinoflagellates was more pronounced during the winter months, particularly August 2001, May 2002 and July 2002.

3.3.4 Ciliate abundance and grazing rates

Ciliate biovolume ranged by a factor of four between 50 000 and 200 000 $\mu m^3\ ml^{-1}$ during all but one of the study periods (Fig. 3.8a). During March 2002 ciliate biovolume was an exceptionally high 464 000 $\mu m^3\ ml^{-1}$. Medium-sized cells dominated ciliate numbers during all months that size class was recorded except July, when small ciliates dominated (Fig. 3.8b).

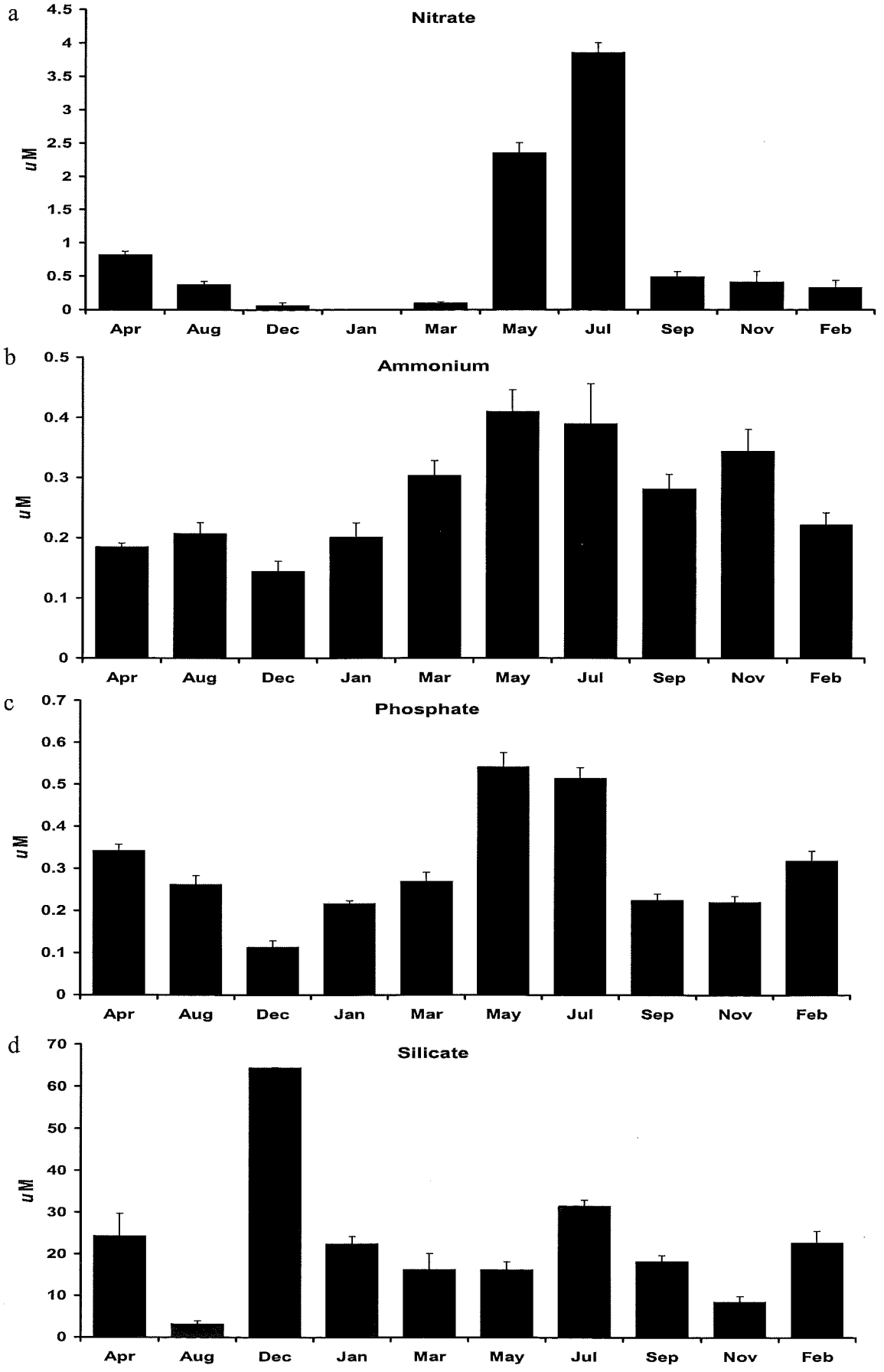


Figure 3.6. Average concentrations of (a) nitrate, (b) ammonium, (c) phosphate and (d) silicate in west Beatrix Bay during sampling periods. Error bars are ± 1 SE.

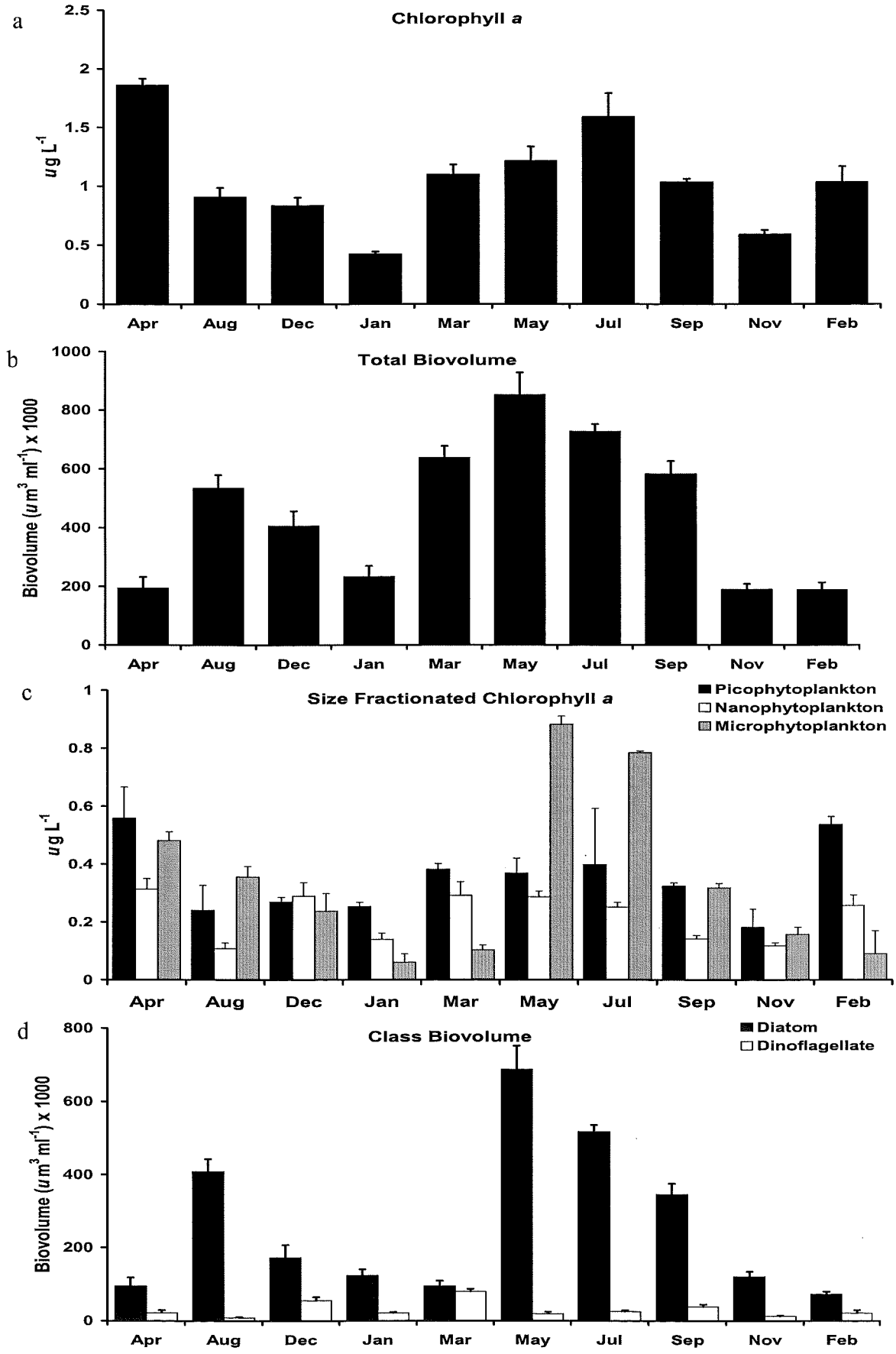


Figure 3.7. Initial phytoplankton levels in west Beatrix Bay during sampling periods. (a) Chlorophyll *a* concentration. (b) Phytoplankton biovolume. (c) Size-fractionated chlorophyll *a* concentration. (d) Diatom and dinoflagellate biovolume. Error bars are ± 1 SE.

Grazing Experiment

The dilution experiment to estimate grazing rates was performed in February 2003 when ciliate biovolume was moderate (93 600 μm^3 ml^{-1}) and was dominated numerically by medium-sized ciliates (Fig. 3.8 a,b). Phytoplankton growth rate exceeded grazing rate in all phytoplankton size classes (Table 3.2). Nanophytoplankton were the most heavily grazed size class of phytoplankton. Grazing rate was greater than 50% of growth rate for this size class ($g:\mu = 0.55$, Table 3.2). Grazing rate was 45% of growth rate for picophytoplankton and only 28% for microphytoplankton. These results suggest that in March 2002, when ciliate biovolume was exceptionally high (five times that of February 2003 - Fig. 3.8a), ciliate grazing rate could have exceeded phytoplankton growth.

3.3.5 Nutrient and shade experiments

Nutrients

Nitrate levels were raised in the nitrate treatments by an average of 5.5 ± 0.3 μM (Table 3.3). The September 2002 experiment (+ 3.4 μM) was the only period when nitrate levels were not raised by at least 5.2 μM above ambient level in the nitrate treatments. This was not thought to affect the results of this experiment, as nitrate levels were still elevated in the nitrate treatments at the end of the experiment.

Chlorophyll a concentration

In general nitrate addition resulted in significantly increased chlorophyll *a* levels ($p<0.001$) (Fig. 3.9, Table 3.4). The effect of nitrate was dependent on the month of the year ($p<0.001$) and the experimental light level ($p<0.05$) (Table 3.4). Chlorophyll *a* concentration significantly increased due to nitrate addition in both the shaded and unshaded treatments in April, August, December, January, September, November, and February (Table 3.5, $p<0.05$). This indicates that the phytoplankton were nitrate-limited during these months. Chlorophyll *a* concentration significantly increased due to nitrate addition in the unshaded treatments only in May (Table 3.5, $p<0.05$). This suggests that co-limitation of nitrate and light may have occurred at this time.

Shading of the microcosms significantly affected chlorophyll *a* levels ($p<0.02$), and this effect was dependent on month and the experimental nitrate level ($p<0.05$) (Fig. 3.9, Table 3.4). Chlorophyll *a* levels were significantly reduced due to shading in both the added-nitrate

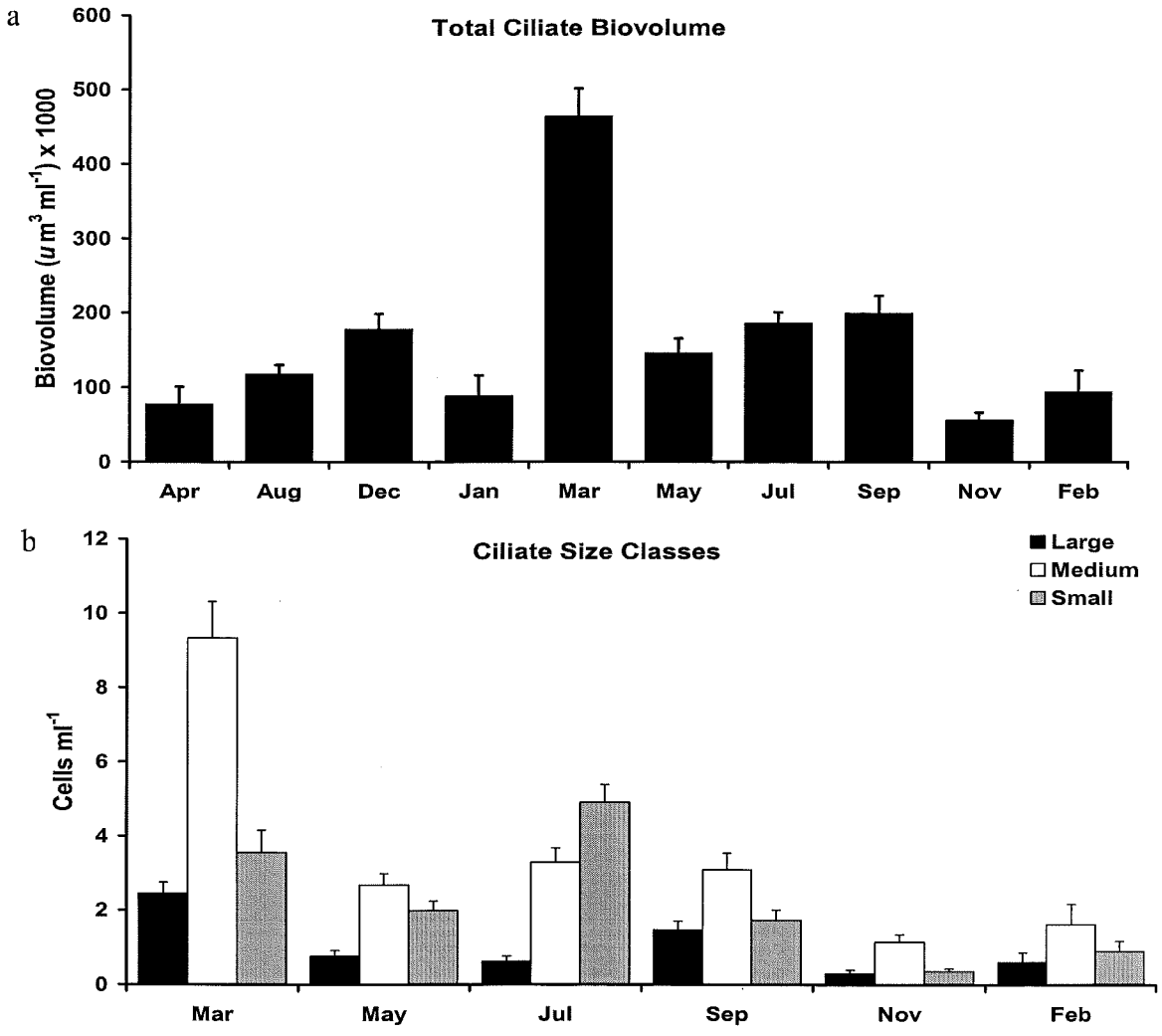


Figure 3.8. (a) Initial ciliate grazer biovolume during sampling periods. (b) Initial ciliate size class structure during sampling periods (from March 2002 onwards). Small < 20 μm in diameter, medium 20 – 50 μm in diameter, large > 50 μm in diameter. Error bars are ± 1 SE.

Table 3.2. Phytoplankton-specific growth rates (μ) and ciliate-specific grazing rates (g) of the different phytoplankton size-classes during the February dilution experiment.

	Microphytoplankton	Nanophytoplankton	Picophytoplankton
μ (d^{-1})	2.93	2.58	1.55
g (d^{-1})	0.81	1.42	0.69
g:μ	0.28	0.55	0.45

Table 3.3. Mean (± SE) nitrate concentration (μM) in experimental treatments on initial (t0) and final (t4) days.

Month	Day	+N	+N/-L	Control	-L
April 2001	t0	6.3 ± 1.1	5.8 ± 0.5	1.0 ± 0.4	0.6 ± 0.02
	t4	0.0 ± 0.0	2.3 ± 0.01	0.2 ± 0.03	0.2 ± 0.02
August 2001	t0	6.0 ± 0.3	5.8 ± 0.3	0.4 ± 0.04	0.4 ± 0.01
	t4	0.4 ± 0.2	3.1 ± 0.5	0.2 ± 0.01	0.2 ± 0.04
December 2001	t0	5.9 ± 0.7	7.1 ± 0.1	0.1 ± 0.01	0.1 ± 0.01
	t4	0.1 ± 0.04	3.0 ± 1.3	0.04 ± 0.01	0.1 ± 0.03
January 2002	t0	7.0 ± 0.4	7.1 ± 0.3	0.1 ± 0.04	0.04 ± 0.0
	t4	0.2 ± 0.2	0.1 ± 0.04	0.1 ± 0.03	0.3 ± 0.1
March 2002	t0	5.2 ± 0.4	5.7 ± 1.1	0.1 ± 0.02	0.1 ± 0.01
	t4	0.7 ± 0.2	5.1 ± 0.8	0.04 ± 0.01	0.02 ± 0.01
May 2002	t0	5.6 ± 1.0	8.2 ± 1.0	1.4 ± 0.2	1.2 ± 0.2
	t4	2.9 ± 0.5	7.5 ± 0.6	0.1 ± 0.01	1.1 ± 0.1
July 2002	t0	7.8 ± 0.04	9.3 ± 1.3	3.2 ± 0.4	3.0 ± 0.3
	t4	6.1 ± 0.9	7.6 ± 1.2	1.5 ± 0.5	1.7 ± 0.1
September 2002	t0	4.4 ± 0.3	4.2 ± 0.4	0.6 ± 0.4	1.2 ± 0.8
	t4	3.6 ± 0.2	4.5 ± 0.7	1.0 ± 0.4	0.4 ± 0.04
November 2002	t0	5.9 ± 0.5	5.5 ± 0.7	0.5 ± 0.3	0.1 ± 0.01
	t4	0.5 ± 0.3	2.7 ± 0.1	0.2 ± 0.1	0.3 ± 0.2
February 2003	t0	6.0 ± 0.6	5.7 ± 0.7	0.2 ± 0.1	0.3 ± 0.2
	t4	0.1 ± 0.01	3.4 ± 1.1	0.1 ± 0.03	0.1 ± 0.02

and ambient nitrate treatments in March, May and July (Table 3.6, $p<0.05$), indicating that the phytoplankton may have been light-limited during this time. Chlorophyll *a* levels were significantly reduced due to shading only in the added-nitrate treatment in April (Table 3.6, $p<0.05$), suggesting that co-limitation of nitrate and light may have also occurred at this time. Chlorophyll *a* levels significantly increased in the shaded treatments in the summer months of December (both treatments), January (added-nitrate treatment only), and February (ambient nitrate treatment only) (Table 3.6, $p<0.05$). This result, which appears to be a response to photoadaptation in the phytoplankton, will be discussed later.

A number of transformations of the data were used but it still failed Cochran's test for homogeneity of variance ($p=0.002$). As indicated in Chapter 2, ANOVA can still operate if variance is heterogeneous when sample sizes are equal. The data were normally distributed.

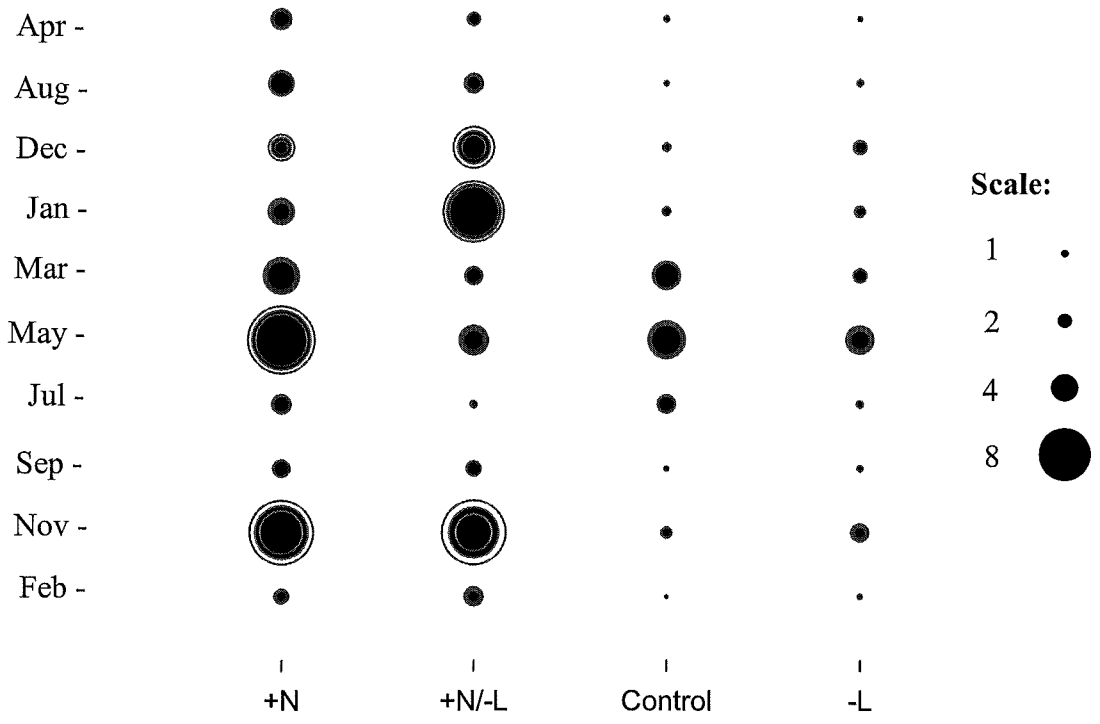


Figure 3.9. Change in chlorophyll *a* in experimental treatments over four days. Change calculated as final concentration/initial concentration. Mean is shown as a black circle. Standard error is shown as a grey ring outside (+) and a grey ring inside (-). Scale shows how the size of the dots relates to the magnitude of the change.

Table 3.4. Summary of 3-way ANOVA. Independent factors are month, nitrate treatment and light treatment. Dependent variable is chlorophyll *a* increase. Asterisks: *p<0.05.

Source	DF	MS	F	*p*
Month	9	2.985	58.10	0.000*
Nitrate	1	22.939	446.51	0.000*
Light	1	0.295	5.75	0.019*
Month*Nitrate	9	0.820	15.97	0.000*
Month*Light	9	0.829	16.14	0.000*
Nitrate*Light	1	0.248	4.82	0.031*
Month*Nitrate*Light	9	0.113	2.21	0.030*
Residual	80	0.051		

Table 3.5. Summary of Student-Newman-Keuls post-hoc test for monthly differences in chlorophyll *a* change in response to nitrate-addition. Significant effect (p<0.05) indicated by an asterisk (*). Underlined asterisk indicates a significant decrease.

Treatment		Apr	Aug	Dec	Jan	Mar	May	Jul	Sep	Nov	Feb
Nitrate-addition	Unshaded	*	*	*	*		*		*	*	*
	Shaded	*	*	*	*				*	*	*

Table 3.6. Summary of Student-Newman-Keuls post-hoc test for monthly differences in chlorophyll *a* change in response to light-reduction. Significant effect (p<0.05) indicated by an asterisk (*). Underlined asterisk indicates a significant decrease.

Treatment		Apr	Aug	Dec	Jan	Mar	May	Jul	Sep	Nov	Feb
Light-reduction	Added-Nitrate	*		*	*	*	*	*			
	Ambient Nitrate			*		*	*	*			*

Total biovolume

There was a significant effect of nitrate addition on phytoplankton biovolume ($p<0.001$), which varied between months ($p<0.001$) (Fig. 3.10, Table 3.7). Biovolume increased significantly in nitrate treatments during August, December, January, November and February (Table 3.8, $p<0.05$). Shading had a significant overall effect on phytoplankton biovolume ($p<0.01$), dependent on month ($p<0.03$). Shading significantly reduced phytoplankton biomass during January, May and July (Table 3.8, $p<0.05$).

Diatom and dinoflagellate biovolume

There was a significant overall response of diatoms to nitrate addition ($p<0.001$) and this response varied significantly between months ($p<0.001$) (Fig. 3.11a, Table 3.9). Diatom biovolume significantly increased in response to nitrate addition during August, December, January, November and February (Table 3.10, $p<0.05$). Dinoflagellate biovolume did not show an overall increase due to nitrate addition (Fig. 3.11b, Table 3.9). January was the only month dinoflagellate biovolume increased significantly due to nitrate addition (Table 3.10). There was a significant overall effect of shading on both diatom and dinoflagellate biovolume (Table 3.9, $p<0.001$). Diatom biovolume decreased due to shading during January, March and May (Table 3.10). Dinoflagellate biovolume decreased due to shading in January and November. Although a variety of transformations were tried on the diatom data, it would not conform to Cochran's test for homogeneity of variance ($p=0.014$). The data was normally distributed however.

A time series of diatom and dinoflagellate biomass ($\mu gC\ l^{-1}$) from 1994 to 2002 shows that diatoms dominated phytoplankton biomass in Beatrix Bay almost all of the time, with the exceptions being occasional dinoflagellate peaks during summer months (Fig. 3.12).

Chain-forming and non chain-forming diatoms

Diatoms were divided into chain-forming and non chain-forming taxa. A significant nitrate effect was found in chain-forming diatoms ($p<0.001$) that varied depending on the time of year ($p<0.001$) (Fig. 3.13a, Table 3.11). Nitrate addition had no significant effect on non chain-forming diatoms (Fig. 3.13b, Table 3.11). Chain-forming diatom biovolume was significantly enhanced in nitrate treatments in August, December, January, November and February (Table 3.12, $p<0.05$). There were no months in which nitrate addition significantly enhanced non chain-forming diatom biovolume.

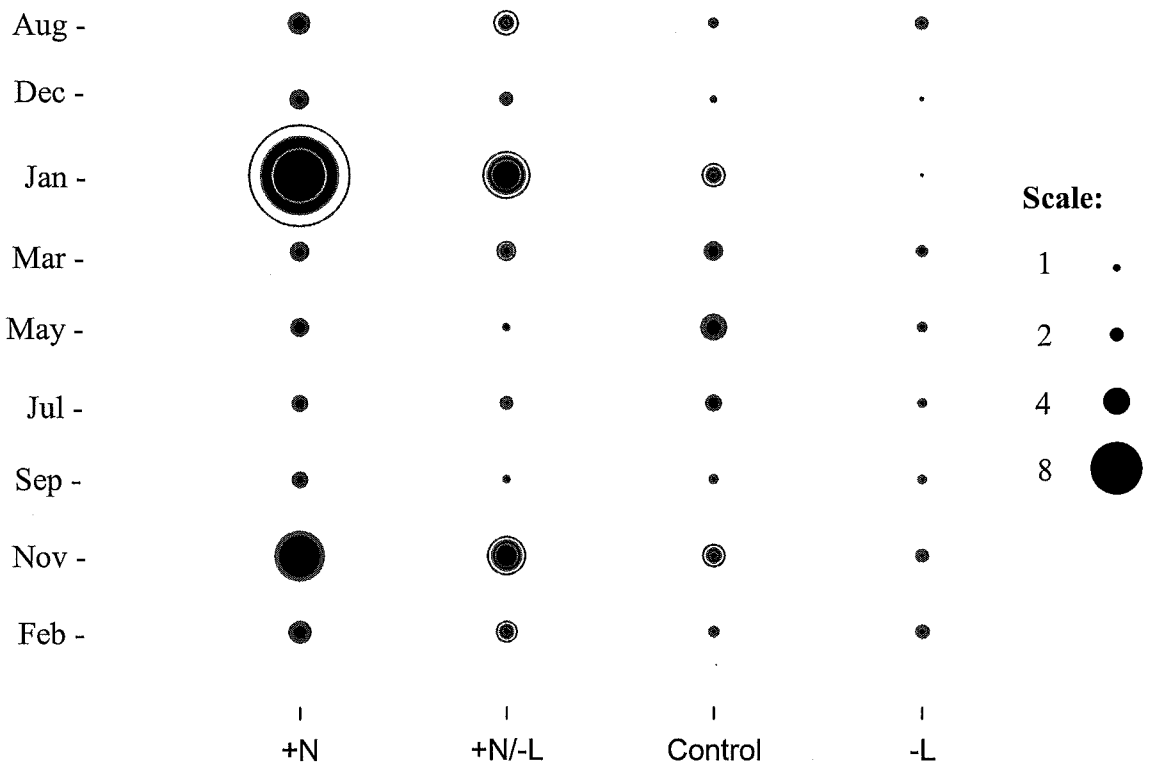


Figure 3.10. Change in phytoplankton biovolume in experimental treatments over four days. Change calculated as final biovolume/initial biovolume. Mean is shown as a black circle. Standard error is shown as a grey ring outside (+) and a grey ring inside (-). Scale shows how the size of the dots relates to the magnitude of the change.

Table 3.7. Summary of 3-way ANOVA. Independent factors are month, nitrate treatment and light treatment. Dependent variable is phytoplankton biovolume increase. Asterisks: *p<0.05.

Source	DF	MS	F	*p*
Month	8	0.030	8.15	0.000*
Nitrate	1	0.221	59.28	0.000*
Light	1	0.108	28.95	0.000*
Month*Nitrate	8	0.037	9.92	0.000*
Month*Light	8	0.009	2.53	0.018*
Nitrate*Light	1	0.000	0.01	0.930
Month*Nitrate*Light	8	0.003	0.92	0.503
Residual	72	0.004		

Table 3.8. Summary of Student-Newman-Keuls post-hoc test for monthly differences in biovolume increase in nitrate and light treatments. Significant effect (p<0.05) indicated by an asterisk (*). Underlined asterisk indicates a significant decrease.

	Aug	Dec	Jan	Mar	May	Jul	Sep	Nov	Feb
Nitrate	*	*	*					*	*
Light			$\underline{*}$		$\underline{*}$	$\underline{*}$			

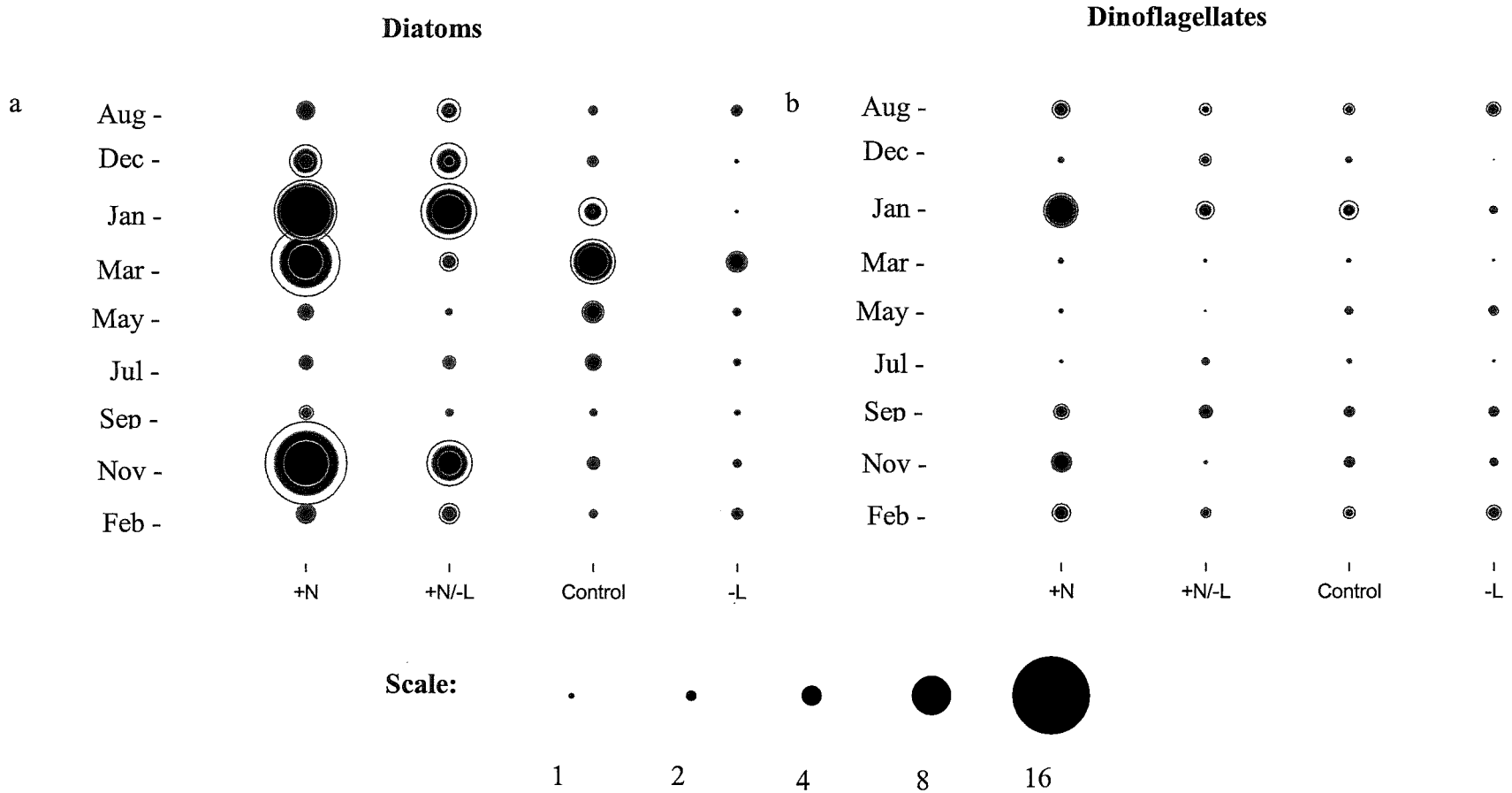


Figure 3.11. (a) Change in diatom biovolume in experimental treatments over four days. (b) Change in dinoflagellate biovolume in experimental treatments over four days. Change calculated as final biovolume/initial biovolume. Mean is shown as a black circle. Standard error is shown as a grey ring outside (+) and a grey ring inside (-). Scale shows how the size of the dots relates to the magnitude of the change.

Table 3.9. Summary of 3-way ANOVA. Independent factors are month, nitrate treatment and light treatment. Dependent variable is diatom and dinoflagellate biovolume increase. Asterisks: *p<0.05.

Source	DF	Diatom Biovolume			Dinoflagellate Biovolume		
		MS	F	*p*	MS	F	*p*
Month	8	0.038	6.18	0.000*	1.182	5.50	0.000*
Nitrate	1	0.315	51.80	0.000*	0.667	3.11	0.082
Light	1	0.156	25.57	0.000*	1.979	9.21	0.003*
Month*Nitrate	8	0.049	8.05	0.000*	0.336	1.56	0.152
Month*Light	8	0.013	2.13	0.043*	0.261	1.21	0.303
Nitrate*Light	1	0.000	0.00	0.976	0.384	1.79	0.185
Month*Nitrate*Light	8	0.008	1.36	0.230	0.301	1.40	0.210
Residual	72	0.006			0.215		

Table 3.10. Summary of Student-Newman-Keuls post-hoc test for monthly differences in biovolume of diatoms and dinoflagellates in nitrate and light treatments. Significant effect (p<0.05) indicated by an asterisk (*). Underlined asterisk indicates a significant decrease.

		Aug	Dec	Jan	Mar	May	Jul	Sep	Nov	Feb
Diatom	Nitrate	*	*	*					*	*
	Light			$\underline{*}$	$\underline{*}$	$\underline{*}$				
Dinoflagellate	Nitrate			*						
	Light			$\underline{*}$					$\underline{*}$	

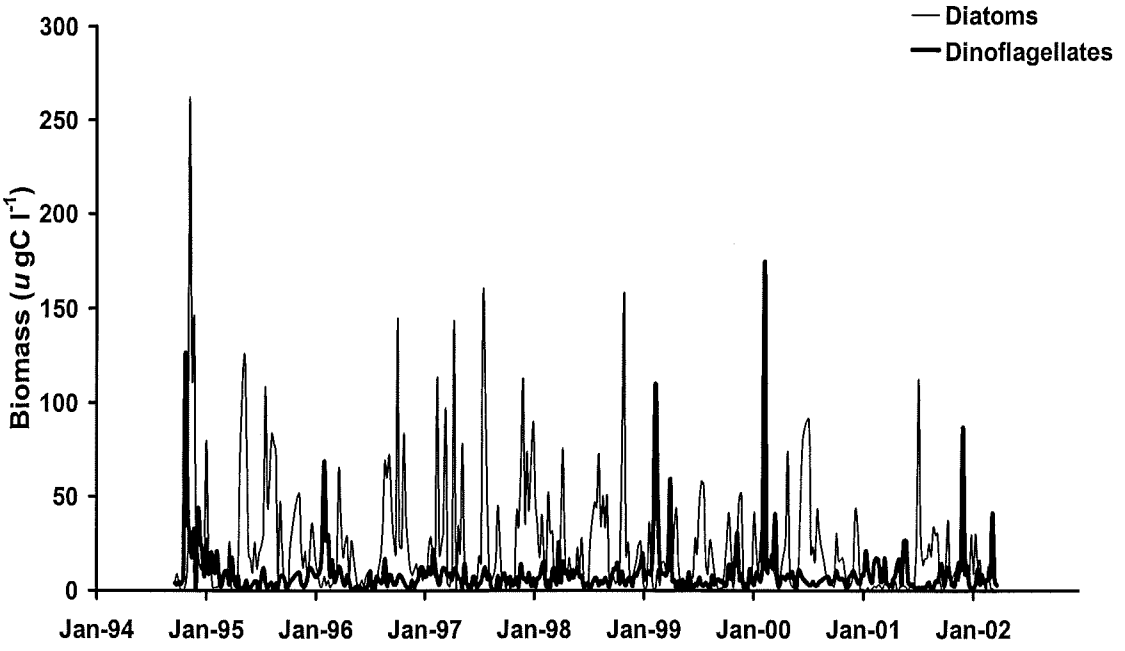


Figure 3.12. Time series of diatom and dinoflagellate biomass in Beatrix Bay from 1994-2002. Data courtesy of NIWA weekly monitoring program. Data represent single weekly depth-integrated samples. For methods refer to Chapter 2.

Shading also had a significant overall effect on chain-forming diatom biovolume ($p<0.001$), and this effect was dependent on the month in which the experiment took place ($p<0.01$) (Table 3.11). Shading significantly reduced chain-forming diatom biovolume in January, March, May and July (Table 3.12, $p<0.05$). Shading had a significant overall effect on non chain-forming diatom biovolume ($p<0.001$) that was not dependent on time of year (Table 3.11). Shading significantly reduced the biovolume of non chain-forming diatoms in August, December, January and March (Table 3.12, $p<0.05$). Despite numerous transformations of the data being tried, non chain-forming diatom data failed the Cochran's test for homogeneity of variance ($p=0.005$). The data were normally distributed.

Chain-forming diatoms dominated diatom biomass in Beatrix Bay most of the time between 1994 and 2002 (Fig. 3.14). The biomass of non chain-forming diatoms rarely exceeded 10 $\mu gC\ l^{-1}$ during this time.

Size classes of diatoms

Diatoms were also divided into size classes. Small size class had a biovolume of 1000 μm^3 ml^{-1} or less, medium size class biovolume ranged from 1000 $\mu m^3\ ml^{-1}$ to 5000 $\mu m^3\ ml^{-1}$, and large size class had a biovolume of more than 5000 $\mu m^3\ ml^{-1}$. Nitrate addition had no significant overall effect on large diatom biovolume (Fig. 3.15a, Table 3.13). January was the only month in which the biovolume of large diatoms significantly increased due to nitrate addition (Table 3.14, $p<0.05$). Medium-sized diatoms responded significantly to nitrate addition ($p<0.001$), and this response was dependent on the time of year ($p<0.001$) (Fig 3.15b, Table 3.13). Medium-sized diatom biovolume significantly increased in response to nitrate addition in August, December, January, November and February (Table 3.14, $p<0.05$). There was also a significant overall increase in small diatom biovolume ($p<0.01$), which was dependent on the time of year ($p<0.03$) (Fig. 3.15c, Table 3.13). Nitrate addition significantly increased the biovolume of small diatoms in January, March and November (Table 3.14, $p<0.05$).

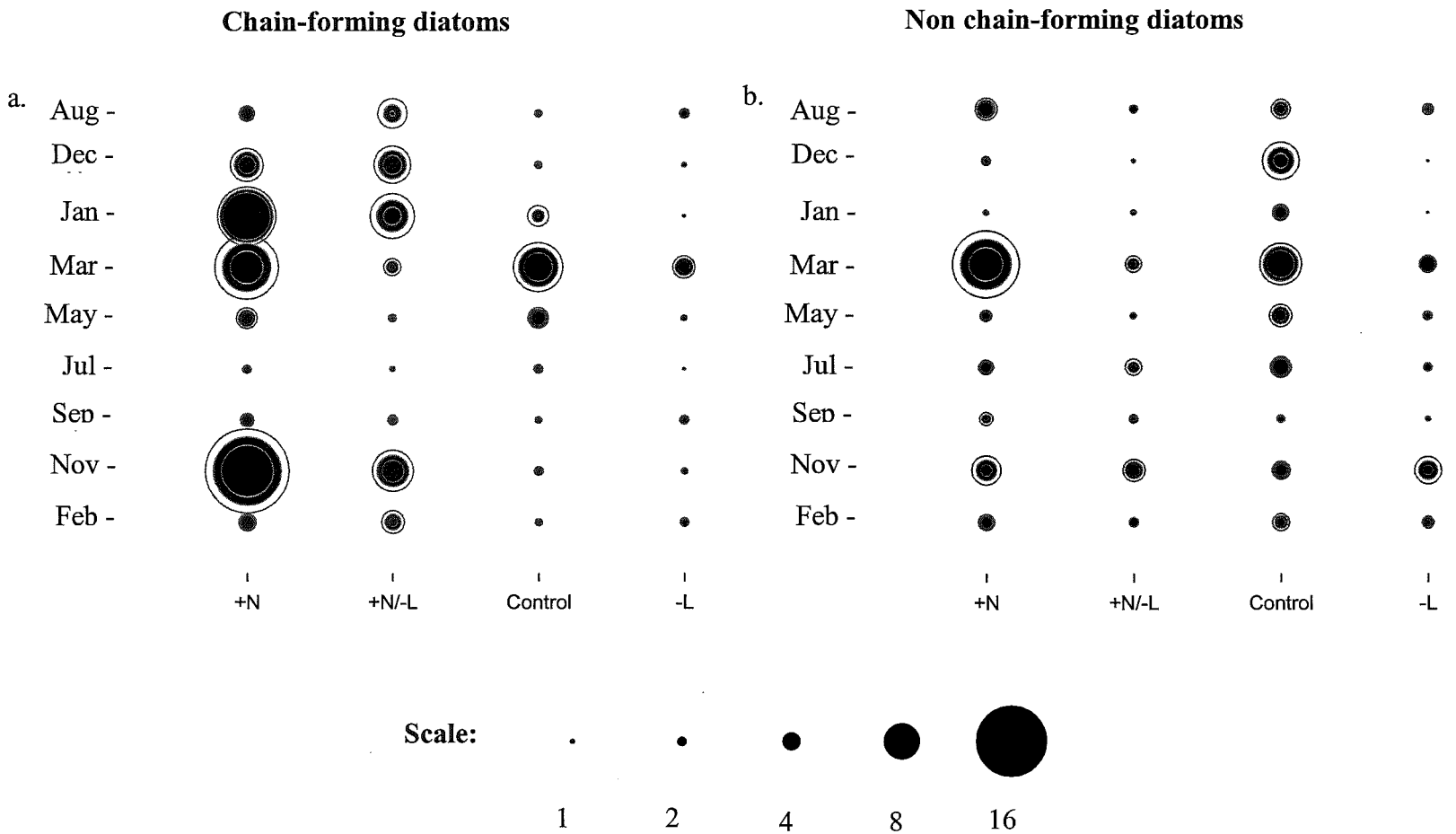


Figure 3.13. (a) Change in chain-forming diatom biovolume in experimental treatments over four days. (b) Change in non chain-forming diatom biovolume in experimental treatments over four days. Change calculated as final biovolume/initial biovolume. Mean is shown as a black circle. Standard error is shown as a grey ring outside (+) and a grey ring inside (-). Scale shows how the size of the dots relates to the magnitude of the change.

Table 3.11. Summary of 3-way ANOVA. Independent factors are month, nitrate treatment and light treatment. Dependent variables are chain-forming diatom biovolume increase and non chain-forming diatom biovolume increase. Asterisks: *p<0.05.

Source	DF	Chain-Forming			Non Chain-Forming		
		MS	F	*p*	MS	F	*p*
Month	8	0.375	5.03	0.000*	0.340	9.67	0.000*
Nitrate	1	4.607	61.77	0.000*	0.040	1.14	0.289
Light	1	1.303	17.47	0.000*	1.217	34.61	0.000*
Month*Nitrate	8	0.444	5.95	0.000*	0.029	0.82	0.592
Month*Light	8	0.246	3.30	0.003*	0.067	1.90	0.074
Nitrate*Light	1	0.063	0.84	0.361	0.114	3.23	0.076
Month*Nitrate*Light	8	0.074	0.99	0.450	0.072	2.06	0.051
Residual	72	0.075			0.035		

Table 3.12. Summary of Student-Newman-Keuls post-hoc test for monthly differences in biovolume of chain-forming and non chain-forming diatoms in nitrate and light treatments. Significant effect (p<0.05) indicated by an asterisk (*). Underlined asterisk indicates a significant decrease.

		Aug	Dec	Jan	Mar	May	Jul	Sep	Nov	Feb
Chain-Forming	Nitrate	*	*	*					*	*
	Light			*	*	*	*			
Non Chain-Forming	Nitrate									
	Light	*	*	*	*					

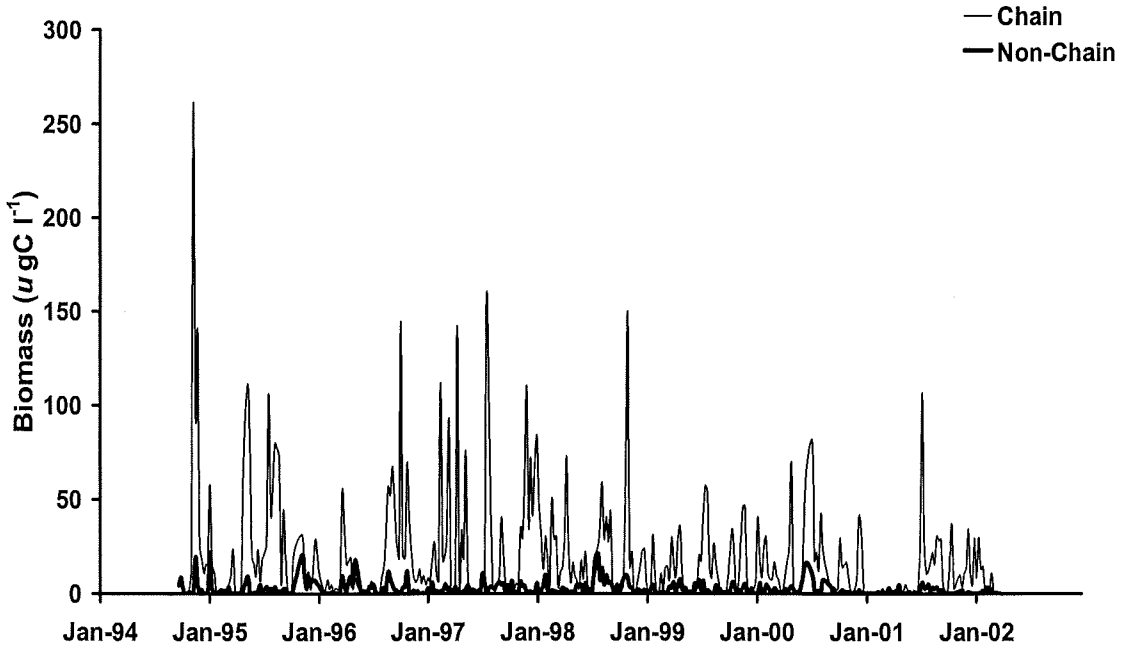


Figure 3.14. Time series of chain-forming and non chain-forming diatom biomass in Beatrix Bay from 1994-2002. Data courtesy of NIWA weekly monitoring program. Data represent single weekly depth-integrated samples. For methods refer to Chapter 2.

Shading had a significant effect on large and medium diatoms ($p<0.05$), and this effect was dependent on month ($p<0.02$) (Table 3.13). Shading caused a significant reduction in the biovolume of large diatoms in December, January, and March (Table 3.14 $p<0.05$). Shading caused a significant reduction in the biovolume of medium-sized diatoms in January, March and May (Table 3.14, $p<0.05$). February was the only month in which shading caused a significant reduction in small diatom biovolume (Table 3.14, $p<0.05$).

The large and medium diatom size class data could not be transformed to pass the Cochran's test for homogeneity of variance ($p=0.003$, $p=0.01$ respectively). Both datasets were normally distributed however.

Medium-sized diatoms dominated diatom biomass in Beatrix Bay between 1994 and 2002, frequently peaking above 50 $\mu gC\ l^{-1}$ (Fig. 3.16). The biomass of small diatoms rarely exceeded 50 $\mu gC\ l^{-1}$, and large-sized diatom biomass never exceeded 50 $\mu gC\ l^{-1}$.

Individual taxa

The response of individual taxa to nitrate addition and shading was also examined. Table 3.15 shows the taxa in order of highest to lowest response to nitrate addition (measured as biovolume increase) according to F-ratio statistics of 3-way ANOVA. Taxa included in the analysis were the most commonly occurring taxa at the beginning of the experimental manipulations. Chain-forming diatoms were the fastest responders. The dinoflagellate taxa tended to be slow responders. Fig. 3.17 shows the time series of the individual taxa ordered from highest responders to nitrate in the top left hand corner to slowest responders in the bottom right corner. The general trend seen is that taxa that showed a high response to nitrate addition have generally maintained higher biomass in Beatrix Bay from 1994-2002 than taxa that did not respond. The exception is *Ceratium* sp., which had two high-biomass peaks early on in the time-series, in 1994-95. There does not appear to be a relationship between taxa abundance and response to shading (Table 3.15).

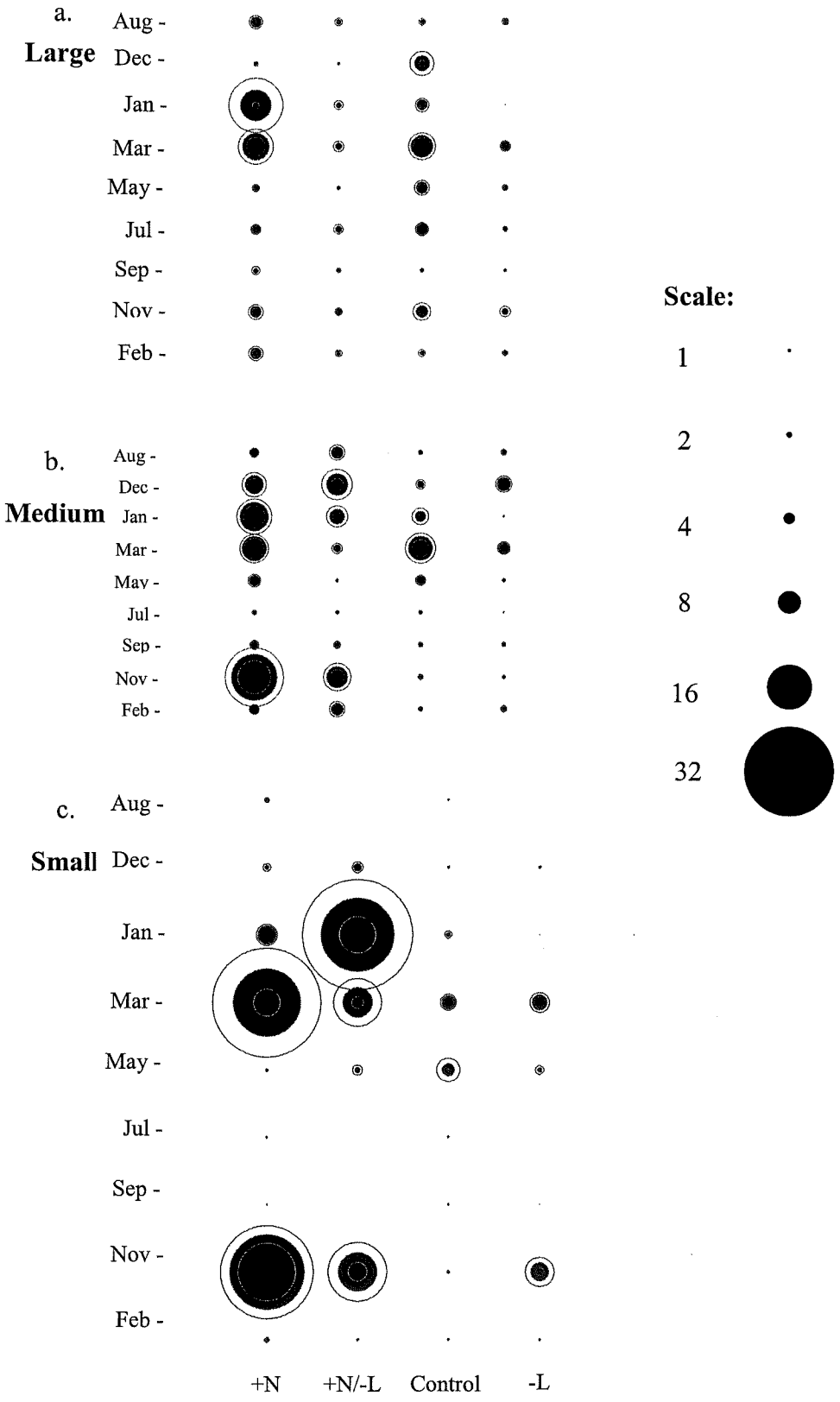


Figure 3.15. (a) Change in large diatom biovolume in experimental treatments over four days. (b) Change in medium diatom biovolume in experimental treatments over four days. (c) Change in small diatom biovolume in experimental treatments over four days. Change calculated as final biovolume/initial biovolume. Mean is shown as a black circle. Standard error is shown as a grey ring outside (+) and a grey ring inside (-). Scale shows how the size of the dots relates to the magnitude of the change.

Table 3.13. Summary of 3-way ANOVA. Independent factors are month, nitrate treatment and light treatment. Dependent variables are change in large diatom biovolume, medium diatom biovolume, and small diatom biovolume. Asterisks: *p<0.05. M = month, N = nitrate, L = light.

Source	DF	Large Diatoms			Medium Diatoms			Small Diatoms		
		MS	F	*p*	MS	F	*p*	MS	F	*p*
M	8	0.056	4.51	0.000*	0.048	8.37	0.000*	1.228	10.74	0.000*
N	1	0.032	2.53	0.116	3.064	53.86	0.000*	2.275	19.90	0.000*
L	1	0.415	33.20	0.000*	1.027	18.05	0.000*	0.158	1.35	0.248
M*N	8	0.017	1.39	0.214	0.334	5.87	0.000*	0.421	3.69	0.001*
M*L	8	0.030	2.36	0.026*	0.288	5.07	0.000*	0.072	0.63	0.751
N*L	1	0.008	0.63	0.430	0.043	0.76	0.385	0.014	0.13	0.723
M*N*L	8	0.016	1.24	0.289	0.067	1.18	0.325	0.208	1.82	0.087
Residual	72	0.013			0.057			0.114		

Table 3.14. Summary of Student-Newman-Keuls post-hoc test for monthly differences in large, medium and small diatom biovolume increase in nitrate and light treatments. Significant effect (p<0.05) indicated by an asterisk (*). Underlined asterisk indicates a significant decrease.

		Aug	Dec	Jan	Mar	May	Jul	Sep	Nov	Feb
Large Diatoms	Nitrate			*						
	Light		*̲	*̲	*̲					
Medium Diatoms	Nitrate	*	*	*					*	*
	Light			*̲	*̲	*̲				
Small Diatoms	Nitrate			*	*				*	
	Light									*̲

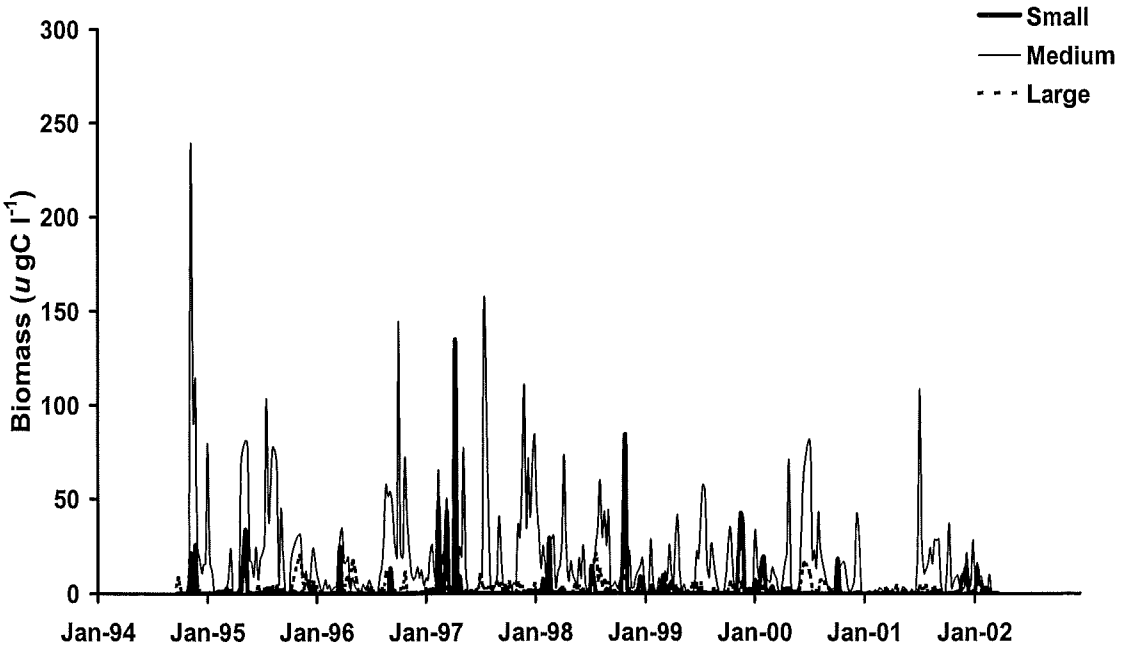


Figure 3.16. Time series of diatom size class biomass in Beatrix Bay from 1994-2002. Data courtesy of NIWA weekly monitoring program. Data represent single weekly depth-integrated samples. For methods refer to Chapter 2.

Table 3.15. F value and p value for individual taxa from 3-way ANOVA. Independent factors are month, nitrate treatment and light treatment. Dependent variable is change in taxa biovolume. Asterisks: *p<0.05. Taxa order from top to bottom is based on response to nitrate (F-value).

Taxa	Month		Nitrate		Light		Month*Nitrate		Month*Light		Nitrate*Light		M*N*L		Class	Type	Size
	F	p	F	p	F	p	F	p	F	p	F	p	F	p			
Chaetoceros sp.	15.1	0.00*	70.2	0.00*	4.9	0.03*	4.8	0.00*	9.0	0.00*	2.9	0.09	1.3	0.27	Diatom	Chain	Medium
Skeletonema sp.	7.1	0.00*	31.9	0.00*	0.5	0.48	9.2	0.00*	1.6	0.16	1.8	0.19	1.5	0.17	Diatom	Chain	Small
Pseudonitzschia sp.	4.7	0.00*	21.1	0.00*	6.8	0.01*	9.0	0.00*	0.5	0.83	0.3	0.60	0.8	0.59	Diatom	Chain	Medium
Thalassiosira sp.	6.4	0.00*	16.2	0.00*	4.4	0.04*	5.9	0.00*	1.8	0.11	5.1	0.03*	0.4	0.90	Diatom	Chain	Medium
Eucampia sp.	3.0	0.01*	11.2	0.00*	2.6	0.12	2.6	0.02*	0.4	0.87	0.3	0.62	0.9	0.50	Diatom	Chain	Medium
Rhizosolenia sp.	12.1	0.00*	8.9	0.00*	2.0	0.16	6.7	0.00*	1.1	0.39	0.7	0.40	1.0	0.42	Diatom	Non-Chain	Large
Cerataulina sp.	3.5	0.00*	6.3	0.01*	2.8	0.10	3.3	0.01*	0.9	0.54	0.0	0.94	0.4	0.90	Diatom	Chain	Medium
Thalassionema sp.	5.9	0.00*	5.8	0.02*	3.5	0.07	3.1	0.01*	3.0	0.01*	4.3	0.04*	0.9	0.52	Diatom	Chain	Medium
Lauderia sp.	17.7	0.00*	5.4	0.02*	2.7	0.10	2.0	0.07	4.1	0.00*	0.6	0.44	2.7	0.02*	Diatom	Chain	Medium
Guinnardia sp.	6.7	0.00*	2.6	0.11	11.9	0.00*	5.1	0.00*	1.5	0.19	0.9	0.36	0.2	0.99	Diatom	Chain	Medium
Scrippsiella sp.	6.4	0.00*	2.6	0.11	11.4	0.00*	1.5	0.20	2.6	0.02*	3.6	0.06	1.7	0.13	Dinoflagellate	Non-Chain	Large
Ditylum sp.	7.5	0.00*	1.9	0.17	10.3	0.00*	1.1	0.36	2.7	0.02*	2.4	0.13	1.2	0.30	Diatom	Non-Chain	Large
Coscinodiscus sp.	2.1	0.05	1.3	0.27	11.9	0.00*	2.7	0.02*	0.8	0.59	1.1	0.30	1.9	0.09	Diatom	Non-Chain	Large
Ceratium sp.	4.1	0.00*	0.7	0.40	0.2	0.63	2.4	0.03*	0.4	0.91	0.1	0.81	2.0	0.07	Dinoflagellate	Non-Chain	Large
Heterocapsa sp.	15.9	0.00*	0.0	0.88	2.1	0.15	4.3	0.00*	2.0	0.07	1.3	0.26	2.8	0.01*	Dinoflagellate	Non-Chain	Medium
Gyrodinium sp.	4.9	0.00*	0.0	0.88	2.0	0.16	1.4	0.24	0.9	0.48	0.5	0.49	0.8	0.62	Dinoflagellate	Non-Chain	Large
Hemiaulus sp.	3.5	0.00*	0.0	0.95	0.9	0.36	0.3	0.97	4.8	0.00*	0.1	0.71	1.7	0.13	Diatom	Chain	Medium
Gymnodinium sp.	2.2	0.05	0.0	0.98	8.7	0.01*	1.3	0.27	1.1	0.35	0.0	0.85	2.2	0.05*	Dinoflagellate	Non-Chain	Medium

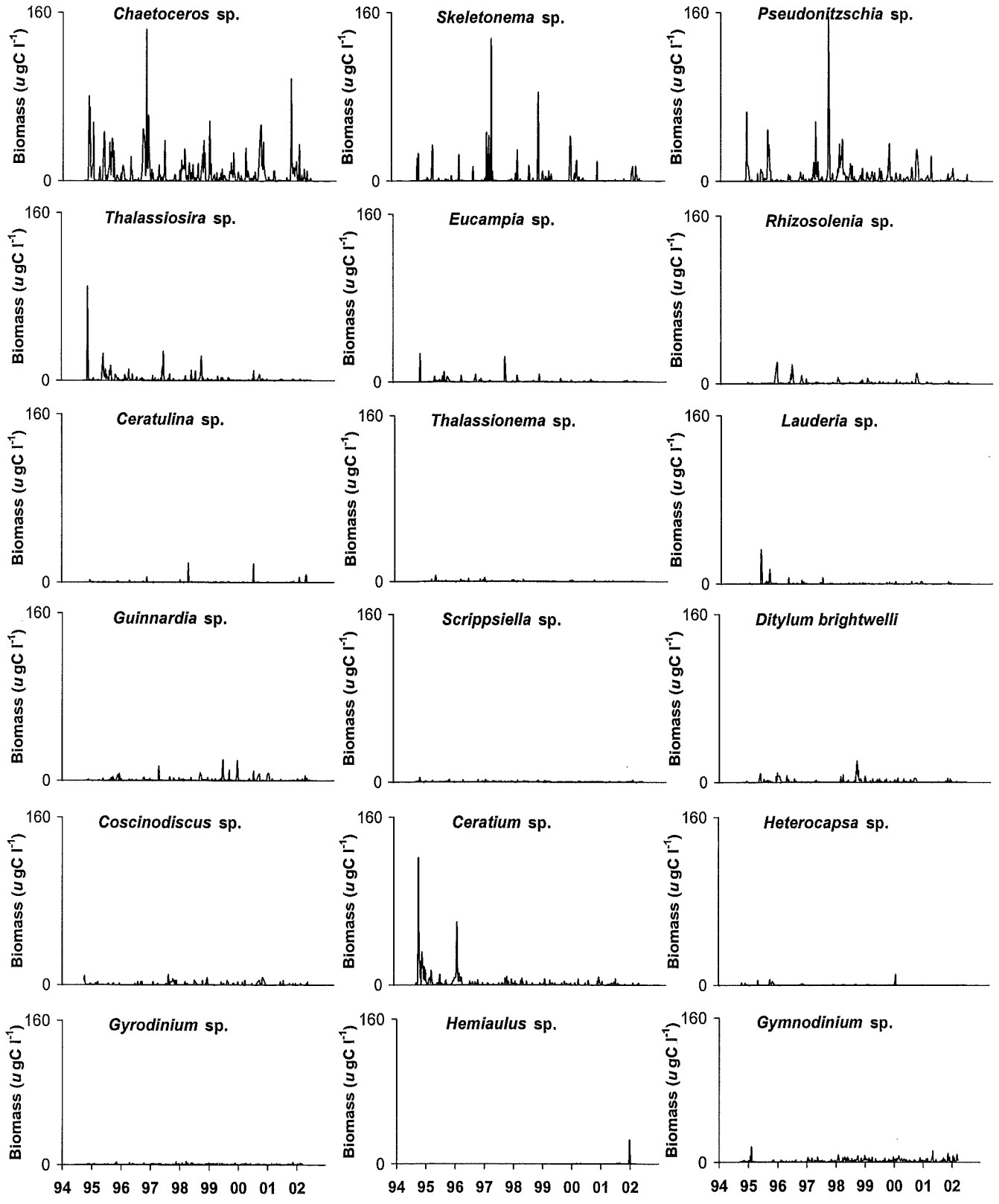


Figure 3.17. Time series of individual taxa biomass in Beatrix Bay from 1994-2002, in order from left to right, top to bottom based on response to nitrate (F-value, Table 3.15) determined from the microcosm experiments. Data courtesy of NIWA weekly monitoring program. Data represent single weekly samples. For methods refer to Chapter 2.

3.4 DISCUSSION

In the introduction I posed seven hypotheses examining the effects of nitrate, light, and microzooplankton grazing on phytoplankton biomass and community structure. This discussion examines the evidence for and against these hypotheses. The Beatrix Bay water column was stratified to some extent during most of the sampling periods. Thermal stratification occurred during spring/summer months when solar irradiance levels were high. The degree of salinity stratification was related to the rate of Pelorus River flow emptying into Pelorus Sound. *In situ* nitrate concentrations showed a similar seasonal pattern to that found by Gibbs and Vant (1997), Ogilvie et al. (2000), and Gibbs et al. (2002), in that nitrate concentrations were high during the winter months but decreased to very low levels during spring and summer. While ammonium and phosphate displayed a similar seasonal cycle of high levels in winter and low levels during spring/summer, concentrations did not vary to the same extent as nitrate. Phytoplankton biomass tended to be higher during winter and early spring, as reported in Gall et al. (2000). This was also a time when diatom biomass was highest. Lowest phytoplankton biomass occurred during summer. Picophytoplankton were not identified by the preservation and microscopic identification techniques used in this study. These tiny cells can comprise a large proportion of total phytoplankton biomass, particularly during summer (Fig. 3.7c).

3.4.1 Phytoplankton response to nitrate addition

During spring and summer months, phytoplankton concentration increased significantly in cubitainers in which nitrate was added, indicating nitrate-limitation of phytoplankton growth at this time (Fig. 3.9, Fig. 3.10). Ambient nitrate levels averaged less than 0.5 μM and ammonium levels less than 0.4 μM during the months a significant nitrate response occurred (Fig. 3.6a, b). Nitrogen limitation of Beatrix Bay phytoplankton growth in spring/summer has also been experimentally demonstrated by Gibbs and Vant (1997) and Ogilvie et al. (2003).

Diatom biovolume increased significantly in response to nitrate enrichment, whereas dinoflagellate biovolume did not (Fig. 3.11). Many studies have shown diatoms to have the fastest phytoplankton growth rates, not only in response to nutrient enrichment (e.g. Latasa et al. 1997; Parsons et al. 1978; Schluter 1998), but also *in situ* (e.g. Furnas 1990). Egge and Aksnes (1992) experimentally showed that when silicate concentrations were above 2 μM,

diatoms could outcompete other phytoplankton groups because they grew faster. Silicate concentration was never below 2 μM during this study (Fig. 3.6d).

The long-term monitoring data from Beatrix Bay indicated that diatoms dominated the phytoplankton biomass between 1994 and 2002, frequently reaching high biomass throughout this time (Fig. 3.12). Conversely, dinoflagellate biomass was consistently low between 1994 and 2002. Dinoflagellate biomass did reach high levels during some summers. With the water column thermally stratified (Table 3.1), and the light levels high (Fig. 3.5b), phytoplankton trapped in the upper water column layer can rapidly exhaust the surface layer of nutrients (Fig. 3.6a) (Margalef 1978). These are conditions in which motile dinoflagellates, that can exploit both the overlying euphotic zone and underlying nutrient-rich waters, can outcompete diatoms (Margalef 1978; Cullen 1982; Mann 1993).

The further division of diatoms into chain-forming and non chain-forming taxa revealed significant differences in their response to nitrate addition. Whereas chain-forming diatom biovolume increased significantly due to nutrient enrichment, non chain-forming taxa did not respond (Fig. 3.13). There has been considerable debate in the literature as to the advantages and disadvantages of chain formation in diatoms. Chain-formation decreases diffusive nutrient supply through a lower surface area-volume ratio (Pahlow et al. 1997), and increases the sinking rate in most species (Munk and Riley 1952; Smayda 1970). It is thought that the main advantage of chain-formation is protection against predation through size-class escape (Munk and Riley 1952). However, increased lateral motion by chain-forming diatoms may increase nutrient absorption. Nutrient uptake by cells can locally deplete the nutrients in the vicinity of the cell (Droop 1973). Chain-forming cells have been shown to undergo increased lateral movement through twisting of the chains, thereby potentially increasing contact with 'new' water (Munk and Riley 1952; Pahlow et al. 1997; Karp-Boss and Jumars 1998).

Chain-forming diatoms characteristically dominate phytoplankton bloom periods when nutrient concentrations are high (Malone 1980; Springer and McCroy 1993; Bode and Dortch 1996). Chain-forming diatoms have dominated the diatom biomass in Beatrix Bay between 1994 and 2002, consistently blooming to high biomass (Fig. 3.14). The biomass of non chain-forming diatoms was consistently low throughout the time-series, never exceeding 20 $\mu gC\ l^{-1}$.

Many studies have shown that smaller size is better in competition for nutrients due to a higher surface area to volume ratio and faster growth rates (Munk and Riley 1952; Eppley and Sloan 1966; Harada et al. 1996). The only factor thought to limit domination by smaller size classes is increased predation on smaller cells (Munk and Riley 1952; Riegman et al. 1993). Therefore, the result that medium-sized cells responded the most often to nitrate addition (Fig. 3.15) was interesting. It is possible that ciliate grazing control of small diatoms limited their apparent growth response. The grazing experiment performed in February demonstrated that nanophytoplankton were the size class that suffered the heaviest ciliate grazing losses. This is the size class that small diatoms would predominantly fall into. Alternatively it may be that other morphological characteristics such as chain-forming or shape are more important than size in determining diatom growth rates in response to nitrate addition.

Medium-sized diatoms, the size class that displayed the most frequent growth increase in response to nitrate addition, dominated diatom biomass in Beatrix Bay between 1994 and 2002 (Fig. 3.16). The biomass of small diatoms, which also showed a significant overall increase to nitrate addition, occasionally peaked above 50 $\mu gC\ l^{-1}$. Large diatoms, which did not significantly respond to nitrate addition, had consistently low biomass throughout the time-series.

The individual taxa that had the largest growth increase in response to nitrate addition were chain-forming diatom taxa such as *Chaetoceros* sp., *Skeletonema* sp., *Pseudonitzschia* sp., and *Thalassiosira* sp (Table 3.15). These were the taxa that consistently bloomed to the highest biomass in Beatrix Bay between 1994 and 2002 (Fig. 3.17). Taxa that did not significantly increase biovolume in response to nitrate addition rarely reached high biomass in the Beatrix Bay time-series. Faster growing taxa have been experimentally shown to have lower nitrogen half-saturation constants (K_s: the nitrogen concentration supporting an uptake rate one-half the maximum rate) (Eppley et al. 1969). An enhanced nitrogen uptake capacity due to a lower K_s value is considered to be an important advantage to species living in nitrogen-limited environments (Eppley and Thomas 1969; Eppley et al. 1969) and environments where nutrient supply is pulsed (Sakshaug and Olsen 1986). Taxa such as *Chaetoceros* sp. and *Skeletonema* sp. have much lower K_s values than taxa such as *Rhizosolenia* sp. and *Coscinodiscus* sp. (Eppley et al. 1969), and this could explain their success in Beatrix Bay, in which nitrate concentration is limiting to phytoplankton growth for

much of the year (Fig. 3.9, 3.10). Nitrate supply is also likely to be pulsed due to the constantly evolving water column stability that is driven by variable freshwater inflow from the Pelorus River for much of the year (Table 3.1, Fig. 3.5a, Stevens 2003).

3.4.2 Phytoplankton response to shading

The two different methods used to estimate phytoplankton biomass, chlorophyll *a* and biovolume, yielded very different results with respect to the shading experiments. Chlorophyll *a* values significantly increased in the shading treatments during the summer months of December, January and February (Fig. 3.9, Table 3.6). However, the biovolume did not change during these months (except for a significant decrease during January) (Fig. 3.10, Table 3.8). This summer increase in chlorophyll *a* levels appears to be a response to photoadaptation, the biological adjustment of phytoplankton pigment production to different light intensities (Lewis et al. 1984). The phytoplankton at this time would have been adapted to high levels of ambient irradiance (Fig. 3.5b). Phytoplankton can adjust to a decrease in light levels during these times by increasing their chlorophyll *a*: biomass ratios in order to increase their light harvesting capacity (Morel et al. 1987; Johnsen and Sakshaug 1993). This photoadaptation of phytoplankton cells can occur within hours (Lewis et al. 1984). Biovolume will therefore better represent changes in phytoplankton biomass when comparing shaded treatments with unshaded treatments, as chlorophyll *a* fluorescence can vary in phytoplankton depending on the light level cells are exposed to (Utkilen et al. 1983; Lewis et al. 1984).

The biovolume results indicate that phytoplankton growth may have been light-limited during January, May and July. Light limitation was expected during the winter months when solar irradiance levels were low (Fig. 3.5b). Gibbs and Vant (1997) and Ogilvie et al. (2003) attributed low growth rates of Beatrix Bay phytoplankton in winter to low light level, and concluded that light was the primary limiting factor to phytoplankton growth at this time. It is puzzling that a significant biovolume reduction occurred in shading treatments during January 2002, a time when solar irradiance levels were high (Fig. 3.5b).

Response to light does not appear to be as important as nitrate in determining taxa abundance in Beatrix Bay. Taxa that had a significant overall reduction in biovolume in shading treatments were both abundant (e.g. *Chaetoceros* sp.) and rare (e.g. *Coscinodiscus* sp.) *in situ* (Table 3.15, Fig. 3.17).

3.4.3 Microzooplankton (ciliate) grazing

During February 2003, ciliate grazing rate accounted for 55% of nanophytoplankton growth rate and 45% of picophytoplankton growth rate (Table 3.2). At this time, *in situ* ciliate biovolume was 93 600 μm^3 ml^{-1}, an average level in Beatrix Bay (Fig. 3.8a). It may have been ciliate grazing that limited the growth response of phytoplankton to nitrate enrichment in the March 2002 experiment. At this time ambient nitrate and ammonium were at limiting levels (0.1 μM and 0.3 μM respectively) (Fig. 3.6a, b), yet a significant growth response to nitrate addition did not occur (Fig. 3.9, 3.10). A factor that may have contributed to the lack of response in March is that 46% of the initial phytoplankton biomass consisted of dinoflagellates (Fig. 3.7d), a group that did not significantly respond to nitrate enrichment in the experiments. However, on the basis of other results, there is an expectation that the remaining 54% as diatoms would have shown a clear biovolume increase in response to nitrate addition at this time. Picophytoplankton and nanophytoplankton size classes, the size classes most susceptible to ciliate grazing, dominated the *in situ* phytoplankton biomass in March. Ciliate biomass was exceptionally high at this time, and it is likely that ciliate grazers removed new cells at a rate equal to or greater than any increase that resulted from nutrient addition.

Microzooplankton have been found to have a large grazing impact on phytoplankton in other systems. James et al. (1996) found that ciliates could potentially graze up to 100% d^{-1} of picophytoplankton biomass in experiments around the South Island. Gallegos et al. (1996) used dilution experiments to demonstrate that at certain times of the year, microzooplankton grazing rates exceeded growth rates for phytoplankton < 5 μm in Manukau Harbour, New Zealand. James and Hall (1998) found that microzooplankton grazing removed up to 92% of phytoplankton standing stock at some stations off the coast of the South Island.

3.4.4 Conclusion

The Beatrix Bay phytoplankton community is comprised of dozens of phytoplankton taxa. Appendix 1 displays a list of taxa, most at genus level, that have been identified in Beatrix Bay. A pattern that consistently emerged out of the enclosure experiments was that taxa with the greatest growth response to nitrate addition were taxa that dominated biomass in Beatrix Bay between 1994 and 2002. Other potentially important factors such as macrozooplankton

grazing, sinking, and light levels were controlled for in these experiments. These results indicate that a key factor structuring the phytoplankton community in Beatrix Bay is the response of taxa to nitrate availability. These are taxa that are not only able to grow rapidly when nitrate is available, but also have a high uptake capacity for nitrate at low concentrations (Eppley et al. 1969). In an environment such as Beatrix Bay where nitrate is often limiting to phytoplankton growth, this would be an important competitive advantage. While peaks in overall biomass in the Beatrix Bay time-series (Fig. 1.5) may not always consist of exactly the same taxa, they tend to consist of taxa with similar morphological and taxonomic traits. Small to medium-sized, chain-forming diatoms dominate peaks in Beatrix Bay phytoplankton most of the time.

CHAPTER 4

Spatial Variability of Beatrix Bay Phytoplankton

4.1 INTRODUCTION

The phytoplankton community in coastal ecosystems is not only structured by temporal changes in nutrient levels, irradiance levels, water column mixing, and grazing pressure; but also by spatial processes. There are a number of physical and biological processes that lead to spatial patchiness in phytoplankton abundance. Phytoplankton have limited motility and therefore horizontal transport driven by tidal currents, wind stresses on the water surface, and horizontal gradients in water density; plays a major role in displacing or mixing water parcels and their phytoplankton (Cloern 1996; Monsen et al. 2002; Martin 2003). The residence time of a coastal ecosystem also has a strong influence on the spatial variability of phytoplankton and nutrient concentrations (Monsen et al. 2002). Cloern et al. (1983) and Relaxans et al. (1988) suggest that blooms can only develop locally if the residence time within the coastal ecosystem is longer than the phytoplankton doubling time. As an extreme example of this, the low plankton abundance in rivers is attributed to the short residence time relative to the population growth rate (Soballe and Kimmel 1987; Basu and Pick 1996). The aim of this chapter was to investigate the spatial processes structuring phytoplankton dynamics in Beatrix Bay and gain an understanding of the extent to which phytoplankton variability is driven by within-bay growth as opposed to advection of cells into the bay.

Both advective processes and within-bay bloom development have been implicated in previous studies on the spatial processes driving blooms. Malone (1977) found that phytoplankton growth rates in the lower Hudson estuary were less than flushing rates and therefore too low to generate blooms. He surmised that increases in phytoplankton biomass must have been due to advection of phytoplankton into the bay. Delmas et al. (1993) concluded that toxic blooms of the dinoflagellate *Dinophysis* spp. occurring in an embayment off the western coast of France, began in the open ocean and were advected in to the embayment. Likewise, it is thought the toxic dinoflagellate *Gyrodinium aureolum* in Norwegian waters blooms offshore, before being advected in to inshore waters (Dahl and Tangen 1993). Red tide blooms of certain dinoflagellate and raphidophyte taxa commonly develop within embayments and estuaries in the Seto Inland Sea of Japan (Honjo 1993) and

along the coast of China (Qi et al. 1993). The development of these blooms is closely linked to nutrient loading of embayment water with industrial and domestic wastewater.

Beatrix Bay is a good system to investigate the spatial processes structuring phytoplankton dynamics due to a number of factors:

- Phytoplankton in Beatrix Bay is nitrate-limited for much of the year (Chapter 3)
- The major source of nitrate to the upper mixed layer in Beatrix Bay is advection via the main Pelorus channel (Gibbs et al. 1992, 2002; Dupra 2000)
- Hydrodynamic exchange between Beatrix Bay and the outside channel varies spatially across Beatrix Bay. This was demonstrated by the tracer simulation of Proctor and Hadfield (1996) (Fig. 1.3). This simulation showed water originating outside the bay mixing along western Beatrix Bay within two tidal cycles. After four tidal cycles water in eastern Beatrix Bay was still water that originated from within the bay at the beginning of the simulation. This indicates that there is more hydrodynamic exchange between western Beatrix Bay and the nitrate-rich water of the main Pelorus channel, and that water in eastern Beatrix Bay has a longer residence time than water in western Beatrix Bay. It was anticipated that the differences in residence time across the bay would provide clues as to the spatial processes structuring phytoplankton within the bay.

The potential for within-bay bloom development to occur in Beatrix Bay was initially tested by comparing the development rate of phytoplankton blooms calculated from the weekly monitoring program data, with the maximum growth rates of these taxa. It was hypothesised that for these blooms to have developed within Beatrix Bay, the maximum growth rate of the taxa must be greater than the development rate observed in Beatrix Bay. This would not prove that a bloom developed within the bay, but would allow for the possibility. If the observed development rate of a bloom within Beatrix Bay exceeded the maximum growth rate of the phytoplankton taxa, advection of cells must have played a role in causing the bloom (possibly in conjunction with growth within the bay).

The differing residence times across Beatrix Bay implies that there is more opportunity for blooms to develop in the more slowly flushed eastern Beatrix Bay than western Beatrix Bay. One would expect within-bay bloom development to be characterised by a higher proportion

of faster-growing taxa in eastern Beatrix Bay. Results from Chapter 3 showed that these taxa were small to medium-sized, chain-forming diatoms. Advection in Beatrix Bay should be characterised by higher nitrate levels in western Beatrix Bay than eastern Beatrix Bay, and a phytoplankton community present that reflects this access to more nitrate. Springer and McRoy (1993) found a plume of nitrate-rich oceanic water advected onto the Bering-Chukchi continental shelf during summer was dominated by chain-forming diatom taxa. These were the taxa that responded most to nitrate enrichment in the enclosure experiments of Chapter 3. Outside the plume, flagellates and slow-growing diatoms dominated biomass.

The hypotheses tested in this chapter were:

- That exchange with the outside channel differs across Beatrix Bay
- That nitrate levels are consistently higher in western Beatrix Bay than eastern Beatrix Bay
- That phytoplankton in eastern Beatrix Bay are more nitrate-limited than phytoplankton in western Beatrix Bay
- That phytoplankton biomass and community composition differs across Beatrix Bay, and is associated with the varying hydrodynamic exchange and nutrient levels across the bay

Surface currents within Beatrix Bay were followed using drogues in order to verify the circulation patterns of Proctor and Hadfield (1996). Study sites were chosen in areas of contrasting hydrodynamic exchange with the outside channel: western, eastern, and outer Beatrix Bay. Sites in western, eastern, and outer Beatrix Bay sampled by the NIWA weekly monitoring program complemented these sites. Environmental variables as well as phytoplankton biomass and community composition were measured and compared at these different sites. Nutrient enrichment experiments were conducted on phytoplankton drawn from western and eastern Beatrix Bay water to test whether nitrate limitation of phytoplankton varied across the bay.

4.2 MATERIALS AND METHODS

4.2.1 Study sites

Samples from bi-monthly sampling trips were collected from three sites: Outer Beatrix, West Beatrix, and East Beatrix (Fig. 2.1). NIWA monitoring program weekly samples were

collected from three sites: outside Beatrix Bay in the main channel (OB); in western Beatrix Bay (WB); and in eastern Beatrix Bay (EB) (Fig. 2.1).

4.2.2 Bimonthly sampling trips

Five-day sampling trips were conducted during the months listed in Table 2.1. Daily samples were taken at the sites West Beatrix, East Beatrix, and Outer Beatrix of water column structure, chlorophyll *a* concentration, nitrate concentration, and phytoplankton taxa. A description of sampling and analysis methods for these variables is in Chapter 2.

4.2.3 Spatial survey

A preliminary spatial survey was conducted in Beatrix Bay in June 2000 during which 10 m integrated samples were taken for determination of nitrate concentration at 25 sites in and around Beatrix Bay (sites shown in Fig. 4.7). The survey was carried out daily over nine days, but due to adverse weather conditions all 25 sites could only be sampled on six of the days.

4.2.4 Weekly Time-Series Sampling

Weekly time-series data for phytoplankton biomass and nutrient concentration are courtesy of the NIWA weekly monitoring program in Beatrix Bay. Methods of sampling and analysis are outlined in Chapter 2. Samples were taken from the sites WB, EB, and OB (Fig. 2.1). Weekly samples were converted to averages for each month of the year for comparison between sites.

4.2.5 Bloom development rates versus maximum growth rates

The development rate of the largest blooms of phytoplankton taxa in Beatrix Bay were calculated from the weekly monitoring data using the equation:

$$\mu = \ln (X_t/X_0)/t$$

where X_t equals the biomass at the peak of the bloom, X_0 equals the initial biomass, and t equals the time in days for the bloom to develop. This was converted to doublings day^{-1} using the equation:

$$\text{doublings day}^{-1} = \mu/0.693$$

This is only an estimate of bloom development rate and would underestimate the rate of development if:

1. the weekly monitoring missed the peak in bloom biomass
2. the bloom began to develop or stopped developing between weekly samples

The maximum growth rates of the relevant taxa from the enclosure experiments of Chapter 3 were calculated using the same growth equations above. Growth rates of taxa from the literature were taken from Furnas' (1990) review of *in situ* marine phytoplankton growth rates. The methods of calculation can be found in Furnas (1990).

4.2.6 Drogues

Drogues, consisting of a large 'sock' suspended vertically in the water column, were used to record current velocity. Drogue surveys were carried out during the April 2001, August 2001, and May 2002 sampling trips. The drogue sock was round, between 1.8 m and 2.2 m in diameter, and 6 m in length (Fig. 4.1). The sock was vertically suspended in the water column from 2 m to 8 m depth. This was designed so that the drogues would follow the current of the surface layer of water only, as the different layers within Beatrix Bay can move with different speeds and directions (Hadfield and Sutton 1996; Sutton and Hadfield 1997). When the water column is stratified in Beatrix Bay the pycnocline is typically below 10 m deep (Hadfield and Sutton 1996; Proctor and Hadfield 1996).

A waterproof-encased Trimble OEM model SK8 GPS that output serial data logged onto a Campbell CR10 Data Logger was attached to the drogues to log the journeys. The Data Logger was programmed to log the position of the drogue every 15 minutes. The drogues were also monitored by boat twice a day and GPS position recorded manually as a backup. Two drogues were used during sampling trips, one launched at the West Beatrix site and one at the East Beatrix site. The drogues were moved back to their site of origin if they moved out of Beatrix Bay or were found beached or caught in a mussel farm.

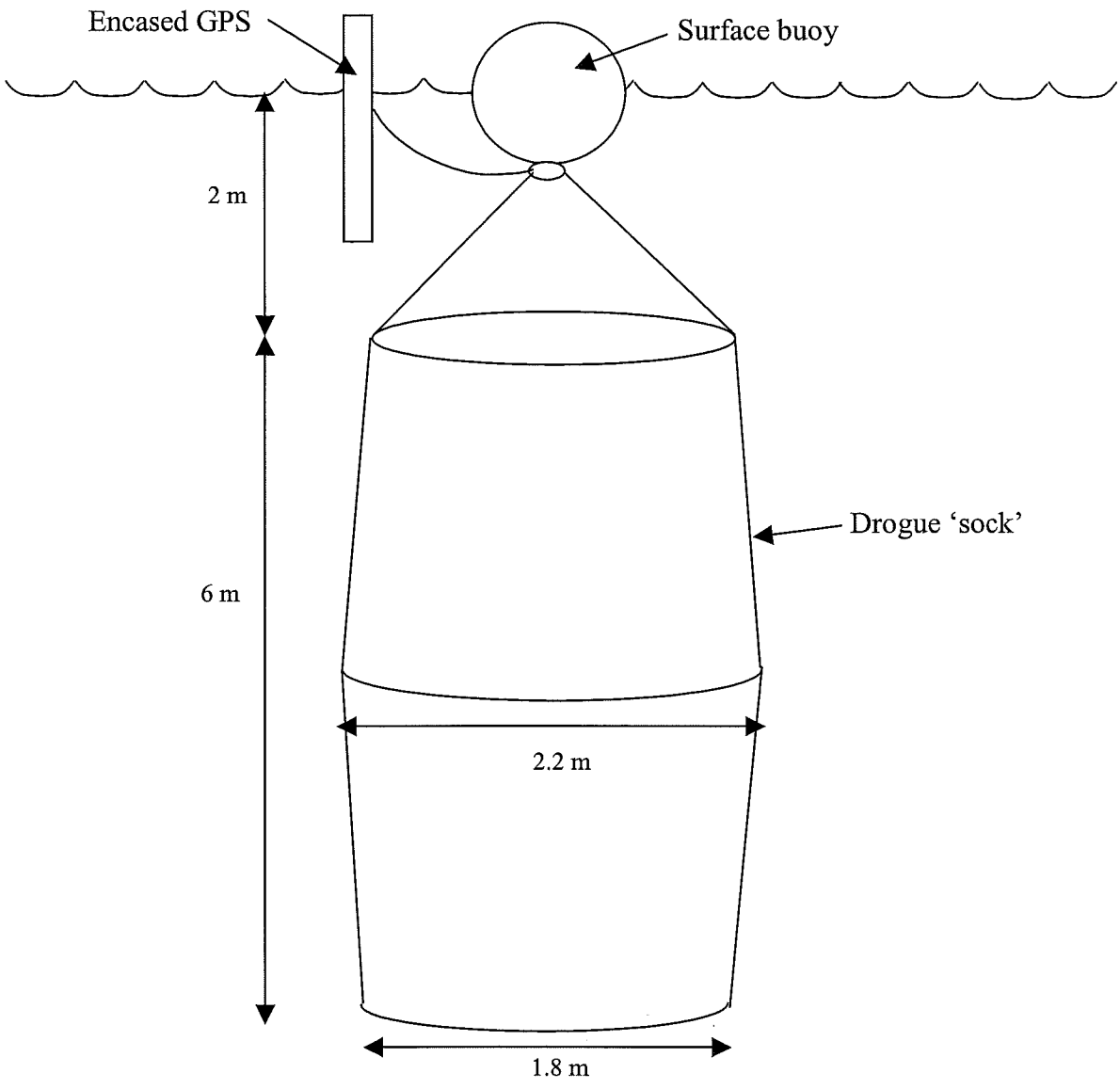


Figure 4.1. Diagram of drogue used to follow water currents. Drogue 'sock' was suspended in the upper mixed layer from 2 m to 8 m depth.

4.2.7 Nitrate addition experiments

In order to test predicted differences in nitrate-limitation of phytoplankton across Beatrix Bay, nitrate addition experiments were conducted in cubitainers using water collected from the sites West Beatrix and East Beatrix. There were two treatments (added-nitrate and control) and two sites (East Beatrix and West Beatrix), with three replicates of each treatment at each site. The experimental set-up is described in Chapter 2. Cubitainers containing water originating from each site were incubated at the same site near the entrance to Clova Bay (Fig. 2.1), to ensure that light and water temperature characteristics were identical for all treatments.

4.2.8 Statistical analyses

To compare the relevant parameters between sites, separate ANOVA tests were used for each sampling trip. ANOVA summary results tables for each sampling trip are displayed in Appendix 4. To compare water column structure between sites, a one-way ANOVA was used with site as the independent factor and density difference (density at 20 m depth minus density at 1 m depth) as the dependent variable. To compare *in situ* nitrate concentration and chlorophyll *a* concentration between sites, two-way ANOVA was used with site and day as independent factors and nitrate concentration or chlorophyll *a* concentration as dependent variables. Student-Newman-Keuls post-hoc tests were used to compare differences among sites. Two-way ANOVA's, with site and treatment as independent factors and chlorophyll *a* increase (calculated final concentration/initial concentration) as the dependent variable, were used for each experiment to compare the response of phytoplankton to nitrate addition between sites.

4.3 RESULTS

4.3.1 Drogues

The logged drogue segments shown (Fig. 4.2) were from the April sampling trip. The drogue launched at West Beatrix tended to move rapidly either further into the bay up the west side, or out of the bay depending on surface currents dictated by tide and wind conditions. The drogue launched at East Beatrix tended to move slowly and remained within eastern Beatrix Bay for days. The purple track in Figure 4.2 represents three days of transport during which time the drogue only travelled to the far entrance of Laverique Bay. The red track represents two days during which the drogue travelled towards the west side of Beatrix Bay before turning back towards the eastern side. This is typical of what was observed. Drogues launched at East Beatrix could often be left for days and remained in the vicinity of eastern Beatrix Bay. Drogues launched at West Beatrix would have to be regularly monitored twice a day having often moved either to the entrance or the foot of the bay in that time.

Figure 4.3a shows drogue 'journeys' when the onboard GPS system failed but the drogue positions were monitored from the boat using a handheld GPS. These journeys were monitored during the April, August and May sampling trips. Again it is evident that drogues launched from West Beatrix would travel along the western side of the bay either to the head or mouth of Beatrix Bay, while drogues launched from East Beatrix would circle within the

eastern eddy. Figure 4.3b shows drogue journeys in which the drogue was found caught within a mussel farm.

4.3.2 Water Column Structure

The density profiles of the water column were not significantly different between sites (Fig. 4.4), with the exception of the March sampling period when East Beatrix had a significantly higher density difference than Outer Beatrix (Student-Newman-Keuls post hoc test, Table 4.1). However, despite this significant result, the water column was weakly stratified at both sites at this time. Beatrix Bay was therefore relatively homogeneous in terms of the vertical structure of the water column.

4.3.3 Nitrate

Bi-monthly intensive sampling

The Outer Beatrix site had the highest nitrate concentration during most sampling periods (Fig. 4.5). Outer Beatrix nitrate concentration was significantly higher than West Beatrix nitrate concentration during four of the nine sampling periods that both sites were measured, and significantly higher than East Beatrix nitrate concentration during five of the nine sampling periods (Student-Newman-Keuls post-hoc test, Table 4.2). Nitrate concentration at West Beatrix tended to be higher than East Beatrix during the winter months (Fig. 4.5, Table 4.2). Nitrate concentrations were similar at East and West Beatrix during the summer months, except for January when nitrate concentration was significantly higher at East Beatrix (Table 4.2).

NIWA monitoring program

Long-term nitrate monitoring revealed a similar pattern in nitrate levels to the bimonthly intensive sampling between the western, eastern and outer areas of Beatrix Bay. Site OB in the main channel had the highest nitrate concentration during all months of the year (Fig. 4.6). From May to October, nitrate concentration tended to be higher at WB than EB. From November to April, nitrate concentration was similar at sites EB and WB.

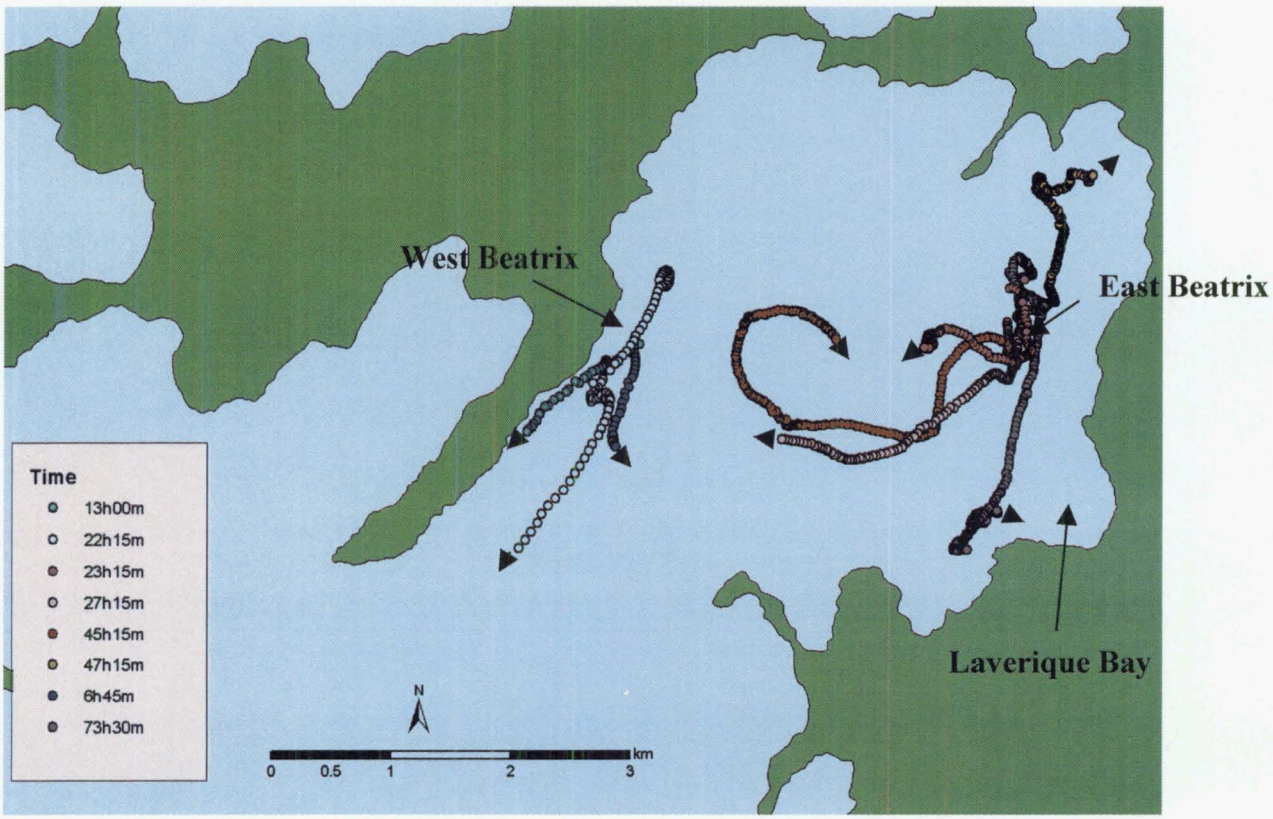
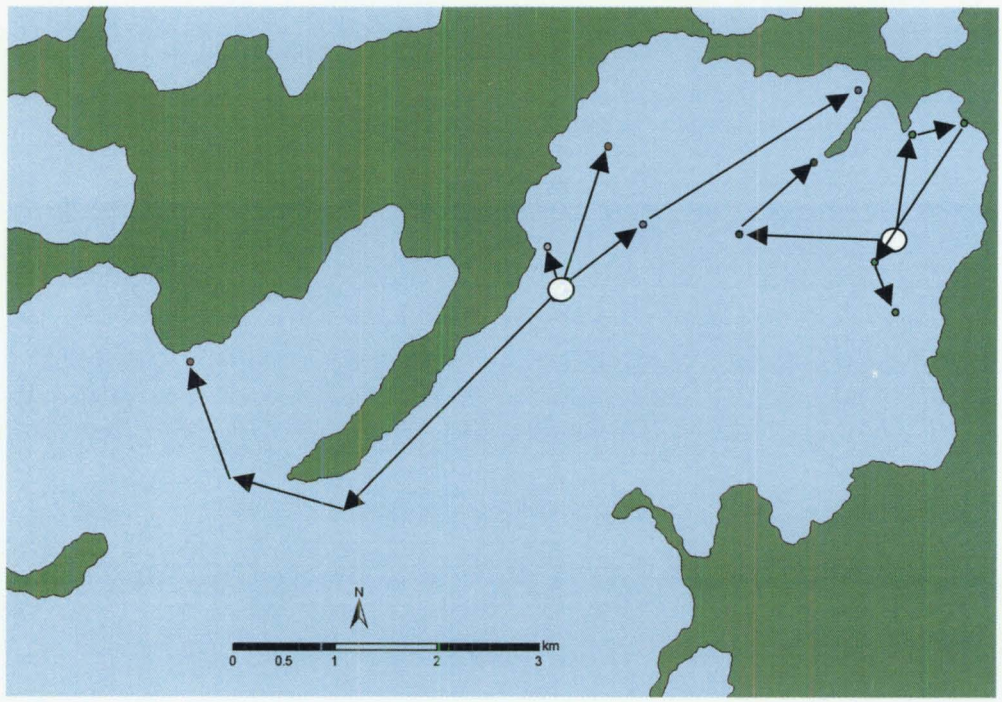


Figure 4.2. Map of Beatrix Bay with drogue tracks recorded from April 2001 field trip. Duration of each track is displayed in the figure legend.

a



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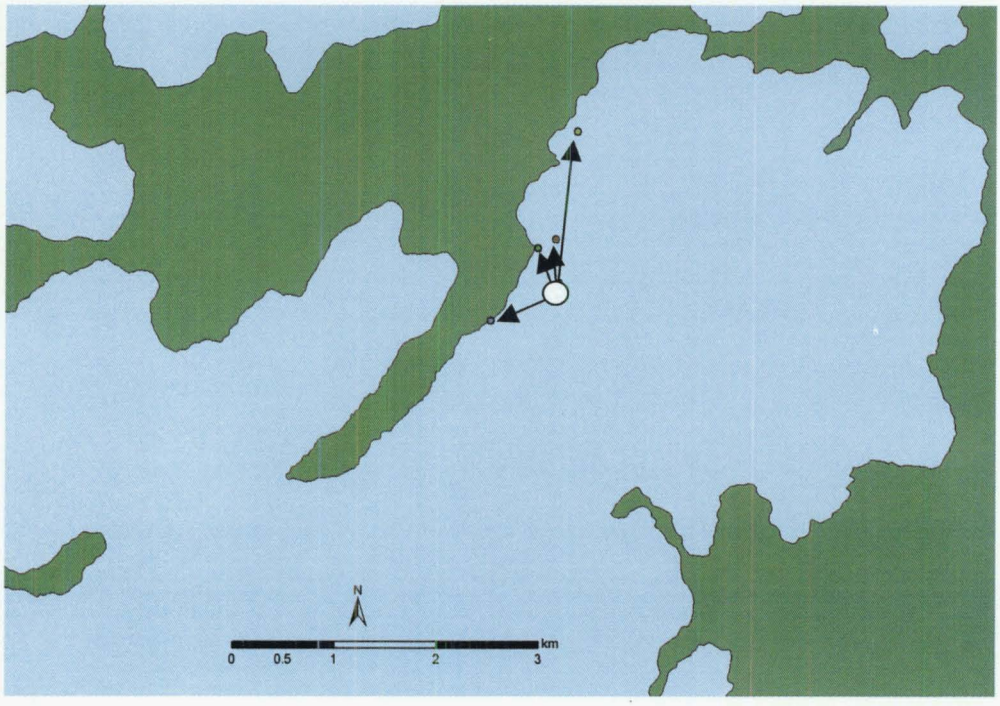


Figure 4.3. a) Map of Beatrix Bay with drogue 'journeys' taken by a handheld GPS when inbuilt GPS system failed. b) Map of Beatrix Bay with drogue 'journeys' that ended with drogue caught in a mussel farm.

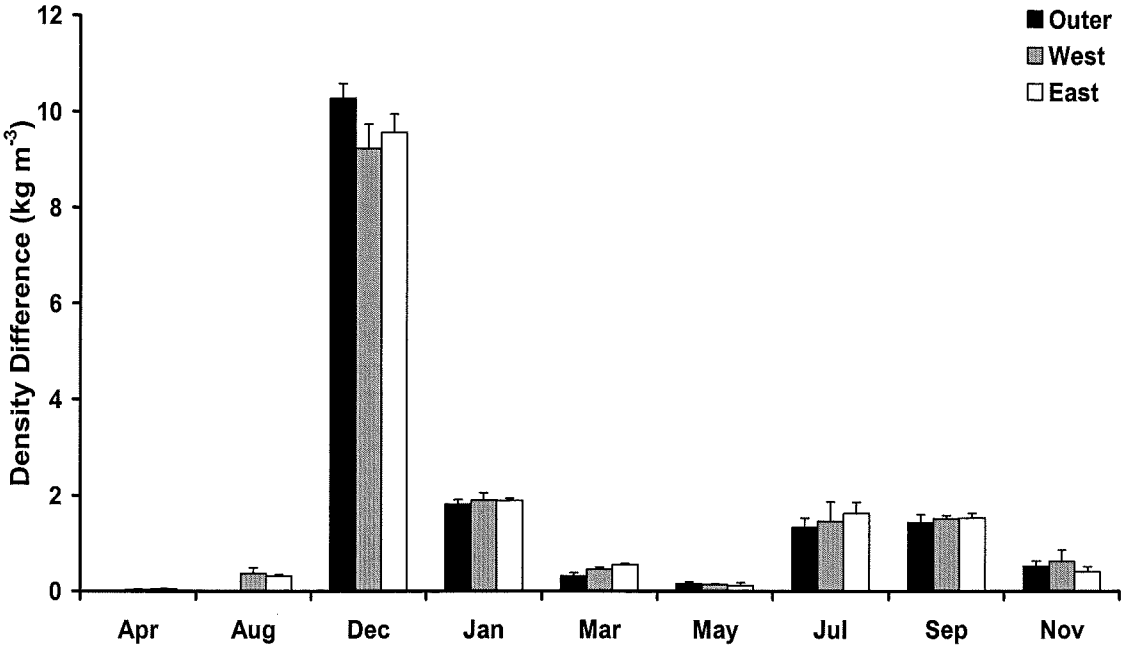


Figure 4.4. Difference in density between 20m depth and 1m depth at three sites in Beatrix Bay. Outer Beatrix site was not measured during April and August. Error bars are ± 1 SE.

Table 4.1. Summary of Student-Newman-Keuls post-hoc test of density differences between sites. One-way ANOVA model was used for each sampling trip with site being the independent factor and density difference the dependent variable. ANOVA summary results tables are displayed in Appendix 4. Density difference calculated as density at 20 m depth minus density at 1 m depth. Significant effect ($p<0.05$) indicated by an asterisk (*). Non-significant effect indicated by NS. Outer Beatrix site was not measured during April and August.

	Outer-West	Outer-East	West-East
April			NS
August			NS
December	NS	NS	NS
January	NS	NS	NS
March	NS	*	NS
May	NS	NS	NS
July	NS	NS	NS
September	NS	NS	NS
November	NS	NS	NS
February	NS	NS	NS

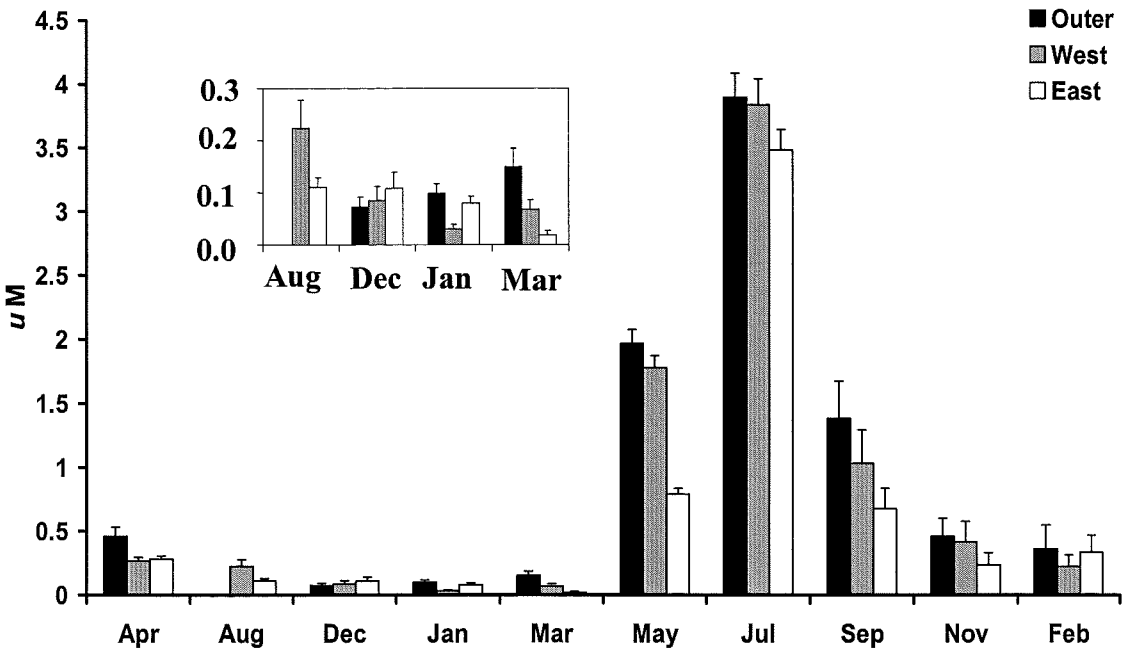


Figure 4.5. Average nitrate concentrations during five day study periods at three sites in Beatrix Bay. Inset shows months when ambient nitrate concentration was less than 0.3 μM. Outer Beatrix site was not measured during August. Error bars are ± 1 SE.

Table 4.2. Summary of Student-Newman-Keuls post-hoc test of differences in nitrate concentration between sites. Two-way ANOVA model was used for each sampling trip with site and day being the independent factors and nitrate concentration the dependent variable. ANOVA summary results tables are shown in Appendix 4. Significantly higher nitrate concentration ($p<0.05$) at first site listed indicated by an asterisk (*). Significantly lower nitrate concentration ($p<0.05$) at first site listed indicated by an underlined asterisk (*). Non-significant effect indicated by NS. Outer Beatrix site was not measured during August.

	Outer-West	Outer-East	West-East
April	*	*	NS
August			*
December	NS	NS	NS
January	*	NS	*
March	*	*	*
May	*	*	*
July	NS	*	*
September	NS	*	NS
November	NS	NS	NS
February	NS	NS	NS

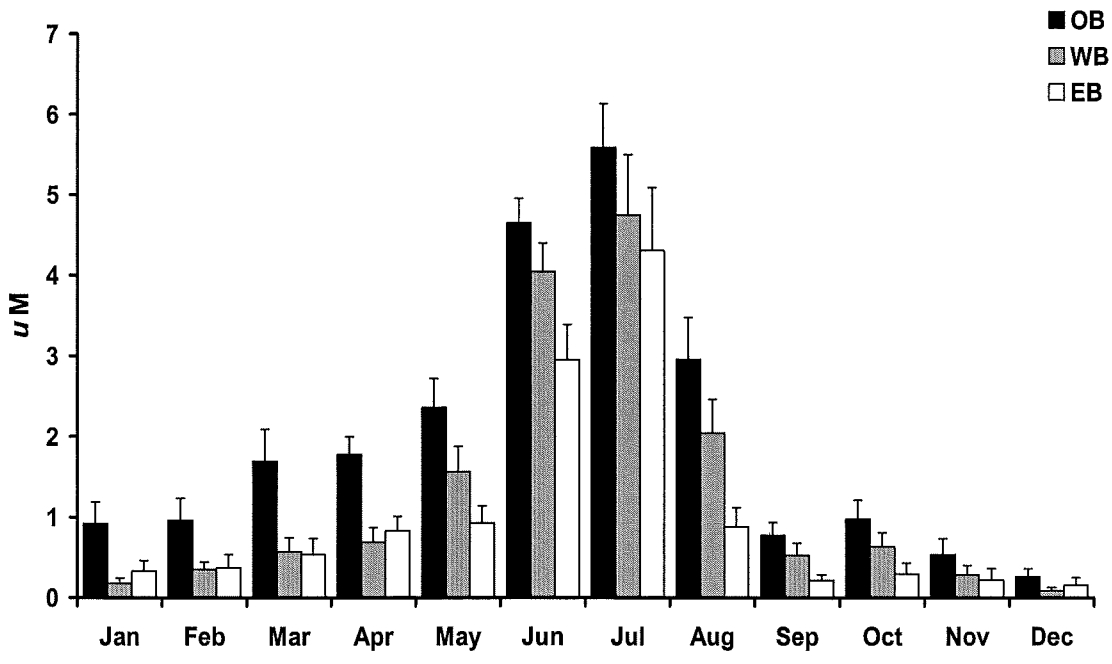


Figure 4.6. Average monthly nitrate concentrations at three sites in and around Beatrix Bay measured between October 1994 and April 2002. Data courtesy of NIWA weekly monitoring program. Error bars indicate standard errors.

Spatial nitrate survey

Circulation in Beatrix Bay is predominantly in and out along the western side, with a slow-moving eddy on the eastern side (Fig. 4.2, Fig. 4.3; Sutton and Hadfield 1997). A spatial survey of Beatrix Bay carried out in July 2000 showed incursions of nitrate-rich water from outside Beatrix Bay into western Beatrix Bay on the 15th and 20th of July (Fig. 4.7). On this basis it can be assumed that the high nitrate concentration in western Beatrix Bay on the 12th resulted from a previous incursion.

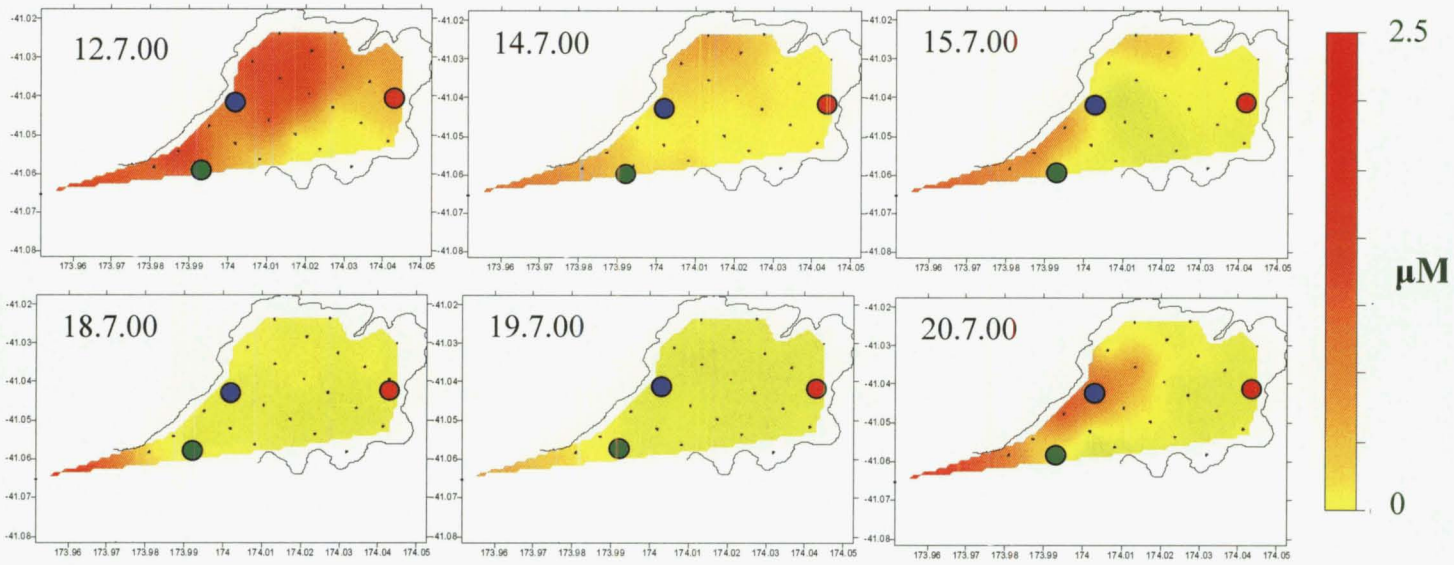


Figure 4.7. Nitrate concentrations, taken from 10 m integrated surface samples, at 25 sites in and around Beatrix Bay. West Beatrix, East Beatrix, and Outer Beatrix sampling sites marked by blue, red and green circles respectively.

4.3.4 Phytoplankton growth rates versus observed bloom development

Maximum calculated growth rates from the cubitainer experiments (Chapter 3) exceeded the observed development rates of taxa blooms in Beatrix Bay for all taxa except *Prorocentrum* sp (Table 4.3). Growth of *Prorocentrum* sp. has been recorded in other studies at 2.0 doublings day^{-1}, exceeding the development rate of 0.81 doublings day^{-1} observed in Beatrix Bay. From these results comparing the development rate of the largest blooms in Beatrix Bay with maximum growth rates of taxa, it was concluded that all phytoplankton blooms observed in the NIWA monitoring data could potentially have developed within Beatrix Bay. Table 4.3 also highlights the disparity in growth potential between diatom and dinoflagellate taxa discussed in Chapter 3. Diatom growth rates from the cubitainer experiments and from the literature are consistently higher among taxa than dinoflagellate growth rates.

Table 4.3. Comparison of observed growth rate of the largest phytoplankton taxa blooms in Beatrix Bay, maximum growth rate from cubitainer experiments (Chapter 3), maximum *in situ* growth rate (where available) from Furnas' (1990) review of *in situ* marine phytoplankton growth rates, and mean *in situ* growth rate (where available) from Furnas (1990). Growth rates as doublings day^{-1}.

Class	Taxa	Observed Beatrix Bay growth rate (doublings day^{-1})	Maximum cubitainer growth rate (doublings day^{-1})	Maximum literature growth rate (doublings day^{-1})	Mean literature growth rate (doublings day^{-1})
Diatoms	*Chaetoceros* sp.	0.83	1.14	3.4	1.4
	Coscinodiscus sp.	0.48	0.68	0.3	
	Ditylum brightwelli	0.35	0.79	2.1	
	Leptocylindricus sp.	0.58	1.12	3.3	1.6
	Pseudonitzschia sp.	0.66	1.16		
	Rhizosolenia sp.	0.72	1.02	4.4	1.2
	Skeletonema sp.	0.91	1.06	5.9	
	Thalassiosira sp.	0.93	1.06	2.0	1.6
Dinoflagellates	*Ceratium* sp.	0.58	0.69		0.27
	Gymnodinium sp.	0.45	0.85	1.7	
	Gryodinium sp.	0.49	0.63	1.6	
	Prorocentrum sp.	0.81	0.55	2.0	0.3

4.3.5 *In Situ* Phytoplankton Biomass

Bi-monthly intensive sampling

West Beatrix and Outer Beatrix tended to have similar chlorophyll *a* levels during sampling trips, whereas East Beatrix chlorophyll *a* levels tended to be significantly different from the other two sites (Fig. 4.8). West Beatrix and Outer Beatrix generally had higher chlorophyll *a* levels than East Beatrix, although they were significantly lower during the April and May sampling trips (Student-Newman-Keuls post hoc test, Table 4.4).

NIWA Monitoring Program

The differences in phytoplankton biomass across Beatrix Bay varied on a seasonal basis (Fig. 4.9). From April to September biomass tended to be highest in eastern Beatrix Bay (EB), intermediate in western Beatrix Bay (WB), and lowest outside Beatrix Bay in the main Pelorus channel (OB). From October to March, biomass tended to be highest outside Beatrix Bay, intermediate in western Beatrix Bay, and lowest in eastern Beatrix Bay. Phytoplankton

biomass outside Beatrix Bay was generally more similar to biomass in western Beatrix Bay than to biomass in eastern Beatrix Bay, reflecting the greater hydrodynamic exchange between western Beatrix Bay and the main channel.

The phytoplankton community composition across Beatrix Bay was investigated by calculating the percentage of total phytoplankton biomass comprising diatoms, dinoflagellates, and chain-forming diatoms. Despite eastern Beatrix Bay having the highest phytoplankton biomass from April to September, there was little difference between sites in the phytoplankton community composition during these months (Fig. 4.10). This pattern was particularly evident from May to August; with all three sites having a similar percentage of diatoms, dinoflagellates, and chain-forming diatoms (Fig. 4.10). From October to March, diatoms generally comprised a greater percentage of overall biomass outside Beatrix Bay, with western Beatrix Bay intermediate, and eastern Beatrix Bay containing the lowest percentage of diatoms (Fig. 4.10). There was a similar pattern with chain-forming diatoms. Dinoflagellates generally comprised a greater percentage of overall biomass in eastern Beatrix Bay at this time, with western Beatrix Bay intermediate, and outside Beatrix Bay tending to have the lowest percentage of dinoflagellates.

4.3.6 Nitrate Addition Experiments

There was a significant increase in chlorophyll *a* in response to nitrate addition in eight of the ten months sampled (Chapter 3). There was a significant difference in response to nitrate addition between East and West Beatrix during three of these months (Fig. 4.11, Table 4.5). During August, September and February a greater growth response to nitrate addition was found in water taken from East Beatrix than West Beatrix. Nitrate levels were lower in East Beatrix than West Beatrix in both August ($p<0.01$) and September (NS) but not in February (Fig. 4.5, Table 4.2).

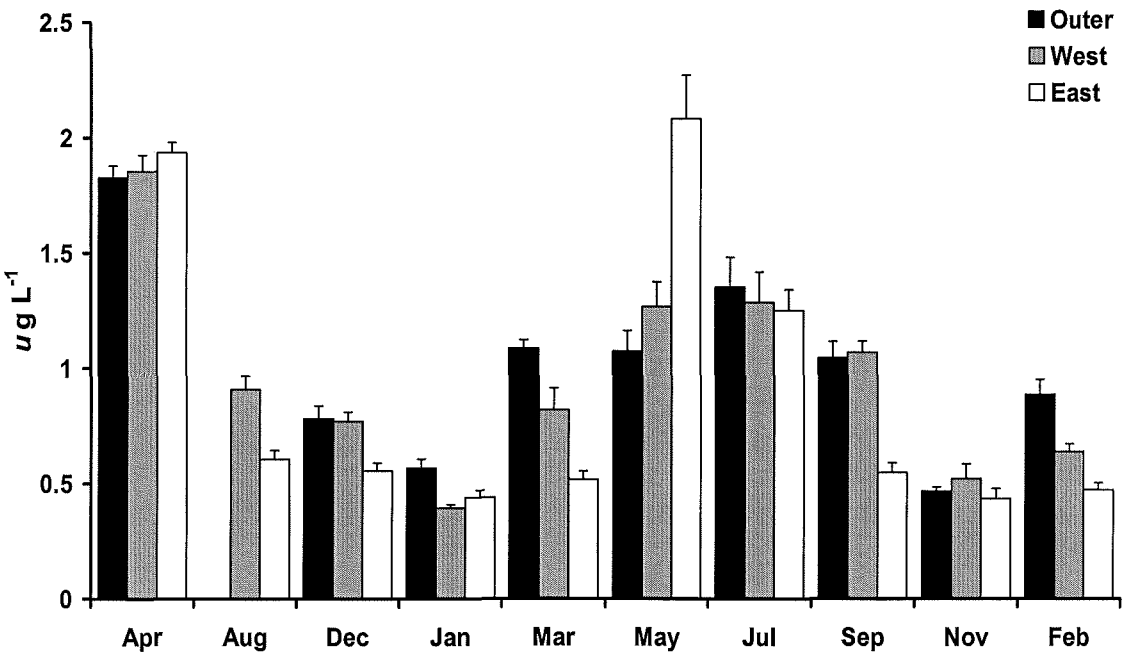


Figure 4.8. Average chlorophyll *a* concentrations during five day study periods at three sites in Beatrix Bay. Outer Beatrix site was not measured during August. Error bars are ± 1 SE.

Table 4.4. Summary of Student-Newman-Keuls post-hoc test of differences in chlorophyll *a* concentration between sites. Two-way ANOVA model was used for each sampling trip with site and day being the independent factors and chlorophyll *a* concentration the dependent variable. ANOVA summary results tables are displayed in Appendix 4. Significantly higher chlorophyll *a* concentration ($p<0.05$) at first site listed indicated by an asterisk (*). Significantly lower chlorophyll *a* concentration ($p<0.05$) at first site listed indicated by an underlined asterisk (*). Non-significant effect indicated by NS. Outer Beatrix site was not measured during August.

	Outer-West	Outer-East	West-East
April	NS	*	*
August			*
December	NS	*	*
January	*	*	NS
March	*	*	*
May	*	*	*
July	NS	NS	NS
September	NS	*	*
November	NS	NS	NS
February	*	*	*

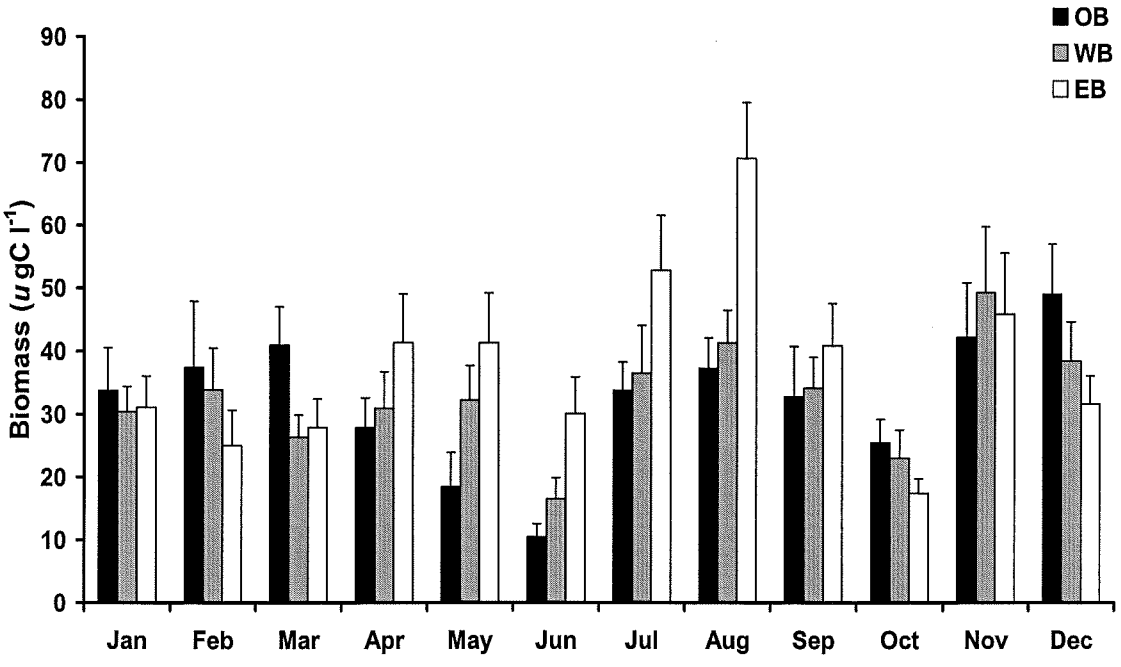


Figure 4.9. Average monthly phytoplankton biomass at three sites in and around Beatrix Bay measured between October 1994 and April 2002. Data courtesy of NIWA weekly monitoring program. Error bars indicate standard errors.

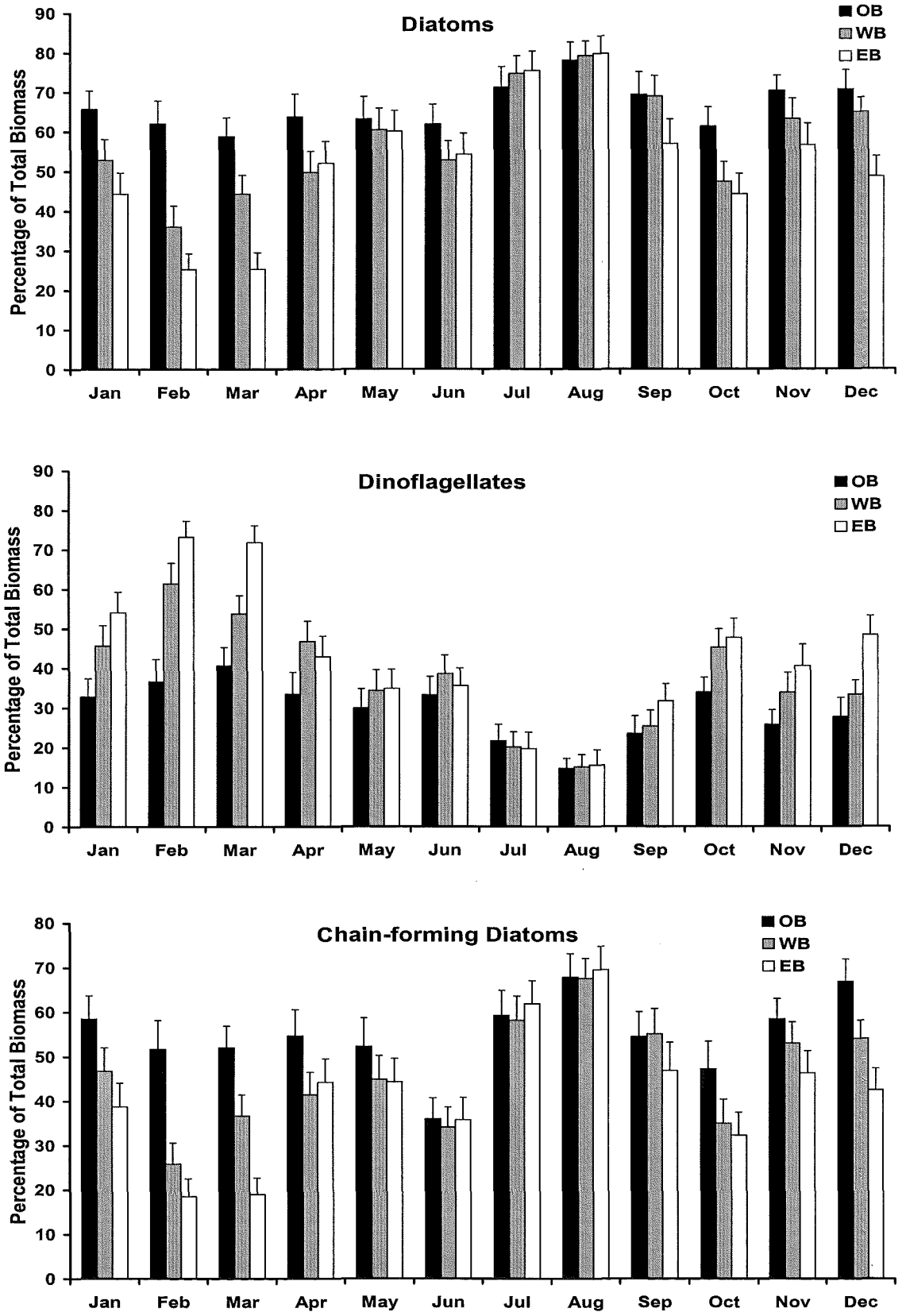


Figure 4.10. Percentage of total biomass comprising diatoms, dinoflagellates, and chain-forming diatoms at three sites in and around Beatrix Bay, measured between October 1994 and April 2002. Data courtesy of NIWA weekly monitoring program. Error bars indicate standard errors.

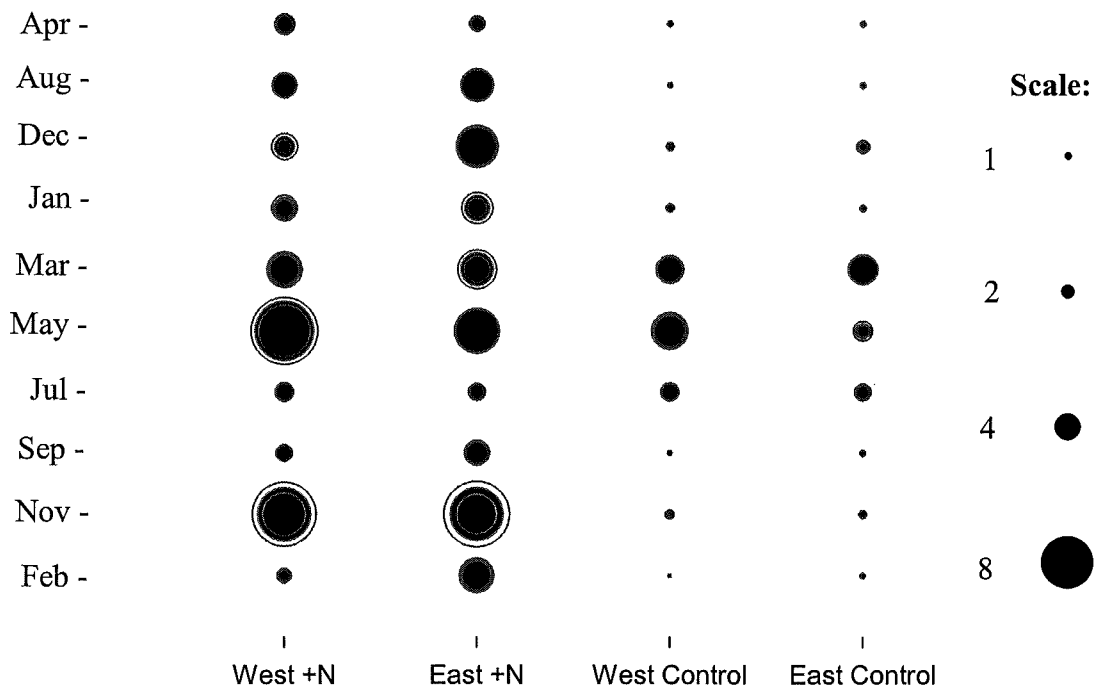


Figure 4.11. Change in chlorophyll *a* in water from West Beatrix and East Beatrix in experimental treatments. Change calculated as final concentration/initial concentration. Mean is shown as a black circle. Standard error is shown as a grey ring outside (+) and a grey ring inside (-). Scale shows how the size of the dots relates to the magnitude of the change.

Table 4.5. Summary of two-way ANOVA's from each experiment with site and treatment as independent factors and change in chlorophyll *a* concentration during the experiment as the dependent variable. Change calculated as final concentration/initial concentration. Site x treatment interaction p-value displayed indicating if the effect of nitrate addition varied significantly between sites. Asterisks: *p<0.05.

Month	p-value
April	NS
August	0.001*
December	NS
January	NS
March	NS
May	NS
July	NS
September	0.003*
November	NS
February	0.000*

4.4 DISCUSSION

In the introduction I posed four hypotheses examining the circulation patterns of Beatrix Bay and how these affect the nutrient levels, phytoplankton biomass and phytoplankton community structure spatially in the bay. This discussion examines the evidence for and against these hypotheses.

4.4.1 Hydrodynamics

Results from the drogue surveys indicated that water tended to get entrained within eastern Beatrix Bay, while the western side of the bay had a much higher hydrodynamic exchange rate with the outer channel. Drogues launched from the West Beatrix site characteristically travelled rapidly along the western side of the bay to the head or entrance of the bay depending on tidal and wind conditions. Upon launching, these drogues needed to be monitored at least twice daily as they could reach the main channel within one day. Conversely, drogues launched at East Beatrix circulated, usually in a clockwise direction, within the eastern side of the bay for days and generally travelled at a much slower speed. This circulation pattern was also evident in results from the spatial grid survey that measured nitrate concentration across Beatrix Bay in July 2000. Incursions of nitrate-rich water could be seen entering western Beatrix Bay from the main Pelorus channel on several occasions (Fig. 4.7).

The observations of higher exchange between western Beatrix Bay and the outside channel, and a clockwise eddy in eastern Beatrix, have been implied in other hydrodynamic studies of the bay. Sutton and Hadfield (1997) found clockwise circulation within Beatrix Bay on some days, with predominantly in and out flow on others in their drogue studies. Proctor and Hadfield (1998) modelled the currents and also found clockwise circulation within Beatrix Bay.

The degree of stratification in Beatrix Bay was found to be relatively homogeneous across the bay (Fig. 4.4). However, in the main channel, where site OB from the NIWA monitoring program is located (Fig. 2.1), the water column is seldom stratified (Gibbs et al. 2002; Stevens 2003). The main Pelorus channel experiences high-energy tidal flows that mix the water column (Stevens 2003). This mixing energy is reduced within embayments, resulting in

stratification of the water column most of the time within Beatrix Bay (Chapter 3). The Outer Beatrix site in this study was located at the entrance to Beatrix Bay (Fig. 2.1), and within the side arm that also contains Crail Bay and Clova Bay (Fig. 1.1). Outer Beatrix is therefore likely to be subjected to reduced tidal flows, and this was reflected in the similarity in water column structure between Outer Beatrix, West Beatrix, and East Beatrix throughout the study period (Fig. 4.4). In contrast, stratification at site OB in the main channel is likely to be significantly reduced (Gibbs et. al. 2002; Stevens 2003).

4.4.2 Growth rates versus observed bloom development

Because the maximum growth rates of taxa exceeded the observed rate of bloom development within Beatrix Bay, it is possible that these blooms developed within the bay. This result does not prove that within-bay development occurred, but merely allows for the possibility. Had the observed bloom development rates exceeded maximum growth rates, it would have meant that advection of cells (possibly in combination with growth) caused the largest of the blooms that occurred in Beatrix Bay between 1994 and 2002.

4.4.3 Physical, chemical, and biological differences across Beatrix Bay

The differences in hydrodynamic exchange between eastern and western Beatrix Bay are important in structuring both the nutrient concentrations and the phytoplankton community. Nitrate is limiting to phytoplankton growth for much of the year (Chapter 3), and most of the nitrate is advected in to Beatrix Bay via the main Pelorus channel (Gibbs et al. 1992, 2002; Dupra 2000). The circulation patterns of the bay mean that the western side of the bay has greater access to nitrate being advected into the bay, as was seen in the spatial grid survey (Fig. 4.7). Because 'older' water tends to become depleted in nutrients due to phytoplankton uptake (Sutton and Hadfield 1997), it was hypothesised that phytoplankton entrained within eastern Beatrix Bay would be more nitrate-limited than phytoplankton in western Beatrix Bay. The nitrate addition experiments indicated that there was more potential for phytoplankton in eastern Beatrix Bay to be nitrate-limited, although this result was not consistent throughout the year (Fig. 4.11, Table 4.5).

Differences were found in both nutrient concentration and phytoplankton community structure between eastern, western, and outer Beatrix Bay. The spatial differences in nutrients and phytoplankton community were seasonally dependent, and can be divided into two 'seasons'. These spatial trends were not as evident during the months bordering these

seasons, probably reflecting a transition phase between the 'summer period' and the 'winter period'.

Summer (Sep/Oct – Apr/May)

The 'summer period' (defined here as Sep/Oct – Apr/May) is the period when nitrate is limiting to phytoplankton growth (Chapter 3). The comparison of phytoplankton and nutrients between outer, western, and eastern Beatrix Bay are summarised in Table 4.6.

Table 4.6. Comparison of phytoplankton and nitrate characteristics between outer, western, and eastern Beatrix Bay during the 'summer period' (Sep/Oct – Apr/May).

	Outer	**West**	**East**
Relative Biomass	Highest	Intermediate	Lowest
Relative Proportion of Diatoms	Highest	Intermediate	Lowest
Relative Proportion of Dinoflagellates	Lowest	Intermediate	Highest
Relative Nitrate Concentration	Highest	Similar	Similar

There is a gradient in phytoplankton biomass and community composition across Beatrix Bay that is related to the hydrodynamics of the bay. Advection of phytoplankton cells from the main Pelorus channel into western Beatrix Bay appeared to be the main structuring influence determining the spatial differences observed across Beatrix Bay at this time. The site outside Beatrix Bay in the main channel had the highest nitrate level and the highest phytoplankton biomass (Table 4.6). This biomass was comprised of a greater percentage of diatoms, a group that thrive when nutrient concentrations are sufficient to permit rapid growth (Chapter 3). The hydro-dynamically isolated eastern Beatrix Bay had the lowest phytoplankton biomass, and a community comprised of a greater percentage of dinoflagellates. Western Beatrix Bay had a higher biomass than eastern Beatrix Bay, with a higher percentage of diatoms. It is logical that this higher biomass of diatoms in western Beatrix Bay originated in the main channel and was advected in. The nitrate concentration at this time is similar in eastern and western Beatrix Bay, despite western Beatrix Bay having greater exchange with the higher nitrate

waters of the main channel. This is likely to be due to the higher biomass of phytoplankton in the stratified, western Beatrix Bay waters rapidly using any additional nitrate advected in at this time. Nitrate can be added to a system with no measurable increase in nitrate levels after a short period of time, if nitrate-depleted phytoplankton rapidly use it up (Venrick et. al. 1987; Mann 1993; Gibbs et al. 2002).

Winter (Apr/May – Sep/Oct)

The 'winter period' (defined here as Apr/May – Sep/Oct) is the period when light tends to be limiting to phytoplankton growth, but nitrate is not (Chapter 3; Gibbs and Vant 1997; Ogilvie et. al. 2003). The comparison of phytoplankton and nutrients between outer, western and eastern Beatrix Bay are summarised in Table 4.7.

Table 4.7. Comparison of phytoplankton and nitrate characteristics between outer, western, and eastern Beatrix Bay during the 'winter period' (Apr/May – Sep/Oct).

	Outer	**West**	**East**
Relative Biomass	Lowest	Intermediate	Highest
Relative Proportion of Diatoms	Similar	Similar	Similar
Relative Proportion of Dinoflagellates	Similar	Similar	Similar
Relative Nitrate Concentration	Highest	Intermediate	Lowest

As nitrate was not limiting to phytoplankton growth at this time (Chapter 3), the isolation of eastern Beatrix Bay from the nitrate-rich water of the main Pelorus channel is not an important factor structuring phytoplankton dynamics across Beatrix Bay in winter. As in summer, there was a gradient in phytoplankton biomass across Beatrix Bay related to the hydrodynamics of the bay. However, in winter biomass was lowest outside Beatrix Bay, intermediate in western Beatrix Bay, and highest in eastern Beatrix Bay.

There is no evidence that the increased biomass in eastern Beatrix Bay from April/May to Sep/Oct was due to phytoplankton blooming whilst being entrained there. The composition of phytoplankton taxa was similar between eastern, western, and outer Beatrix Bay. Due to the longer residence time of the eastern Beatrix Bay, it was hypothesised that within-bay bloom

development would be characterised by a greater proportion of rapidly growing taxa in eastern Beatrix Bay. The higher growth potential of diatom taxa (and especially chain-forming diatom taxa) compared with dinoflagellate taxa is evident from both the enclosure experiments of Chapter 3 and the literature (Table 4.3). However, phytoplankton composition was similar in eastern Beatrix Bay to western Beatrix Bay and outer Beatrix Bay between April and August (Fig. 4.10). In September diatom percentage was lowest in eastern Beatrix Bay, the opposite of what would be expected if bloom development were occurring within Beatrix Bay.

It is apparent from the literature that embayments where within-bay bloom development has been reported often have high levels of anthropogenic eutrophication inputs (e.g. Paerl 1988; Honjo 1993; Qi et al. 1993). For example, Mukai (1987) studied the spatial heterogeneity of phytoplankton and physical and chemical variables in Hiroshima Bay, a large embayment with a surface area of about 946 km^2, a residence time of approximately 76 days, and marked eutrophication in some areas due to anthropogenic inputs. The phytoplankton community varied spatially and this variation was found to be due to phytoplankton growth within the bay responding to marked differences in environmental variables. However, the size, residence time, and scale of difference in environmental variables across Hiroshima Bay mean it cannot be reasonably compared with Beatrix Bay. There is no such source for nutrients within Beatrix Bay. Ogilvie et al. (2000) found enhancement of phytoplankton growth within mussel farms during summer months, and concluded this was because mussels, which are producers of dissolved inorganic nitrogen, were supplementing low ambient nitrogen levels. However, measurements in the study were taken within a few metres of mussel dropper ropes, and this effect was considered to be highly localised.

Instead it appears that the spatial differences across Beatrix Bay in winter are due to water in western Beatrix Bay being 'diluted' with low biomass water advected in from the main channel. This low phytoplankton biomass in the main channel during winter can be attributed to light-limitation of phytoplankton at a time when irradiance is low (Fig. 3.5b). The lack of stratification in the main Pelorus channel (Gibbs et al. 2002; Stevens 2003) means that phytoplankton will be mixed deeper (and therefore receive less light) than cells within Beatrix Bay, where the water column is usually stratified (Fig. 3.4). The increased exchange between this low biomass water in the main channel and western Beatrix Bay results in a

biomass that is lower in western Beatrix Bay than eastern Beatrix Bay at this time. It is likely that the lower nitrate concentrations in eastern Beatrix Bay at this time were due to the higher biomass of phytoplankton present utilising nitrate.

4.4.4 Conclusion

There are spatial differences in both nutrients and phytoplankton across Beatrix Bay that vary seasonally but appear to be related to the hydrodynamics of the bay. Advection of water into western Beatrix Bay plays a major role in the spatial variation across Beatrix Bay throughout the year. During the summer period, when nitrate is limiting to phytoplankton growth, biomass is highest outside Beatrix Bay where phytoplankton have access to higher nutrient concentrations. Advection of this high biomass water into western Beatrix Bay results in a higher biomass in western Beatrix Bay than eastern Beatrix Bay at this time. During winter, light is limiting to phytoplankton growth, and biomass is lowest outside Beatrix Bay where phytoplankton are mixed deeper into the water column. The increased exchange between this low biomass water in the main channel and western Beatrix Bay results in a biomass that is lower in western Beatrix Bay than eastern Beatrix Bay at this time.

CHAPTER 5

Factors Driving Long Term Phytoplankton Dynamics: Influence of El Niño-Southern Oscillation

5.1 INTRODUCTION

This chapter investigated the processes and mechanisms driving interannual variation in phytoplankton community dynamics in a coastal ecosystem. A time series of diatom and dinoflagellate biomass in Beatrix Bay shows variability in abundance at interannual time scales (Fig. 5.1). While dinoflagellate biomass tended to peak during summer, biomass peaks were suppressed during the summers of 1994-95, 1996-97, 1997-98, and 2000-01. Diatom biomass was highest during 1995 and from mid 1996-1998. The aim of this chapter was to determine whether interannual variability in Beatrix Bay phytoplankton is influenced by large-scale climatic variability.

Climatic variability in New Zealand at annual to decadal time-scales is principally driven by the El Niño-Southern Oscillation (ENSO) phenomenon (Brenstrum 1998). This involves aperiodic exchange in air pressure between the Indonesian area and the south-east Pacific. ENSO is quantified by the Southern Oscillation Index (SOI), a measure of differences in air pressure between Tahiti and Darwin. These differences in air pressure alter the intensity and direction of the Pacific trade winds. El Niño events occur approximately every four years (Hansen 1990), and are characterised by unusually warm sea surface temperature (SST) off the western coast of South America and low rainfall in the Indonesian area of the western Pacific. The opposite condition, La Niña, is characterised by colder than normal SST's off the western coast of South America. ENSO has been linked to global scale interannual climate variability (Burroughs 1992).

The specific hypotheses tested were:

- That ENSO affects Beatrix Bay phytoplankton through climate-driven changes in upwelling in Cook Strait, thereby altering nitrate availability to Beatrix Bay at interannual time scales

- That ENSO affects Beatrix Bay phytoplankton through climate-driven rainfall anomalies altering Pelorus River flow, thereby affecting the degree of salinity stratification in Beatrix Bay at interannual time scales

The relationships between phytoplankton species and relevant environmental variables between 1994 and 2003 were examined. During this period, ENSO fluctuated between El Niño and La Niña phases several times. Included in this time-series was the 1997-1998 El Niño event, considered by some measures the strongest of the 20th century (Brenstrum 1998; McPhaden 1999).

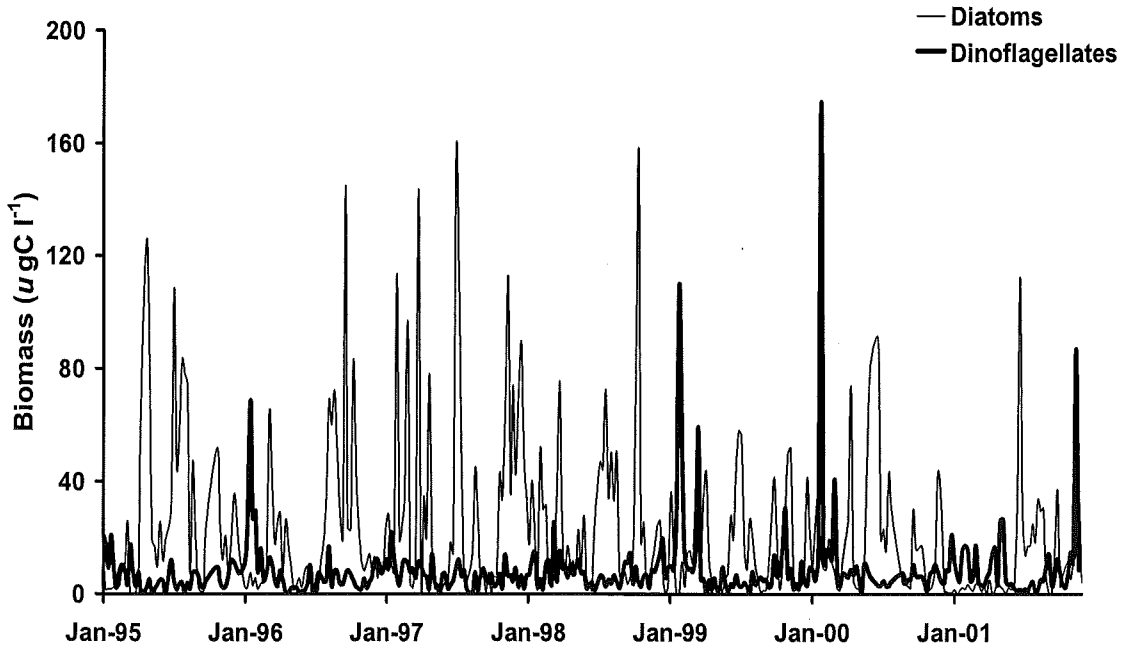


Figure 5.1. Diatom and dinoflagellate biomass in Beatrix Bay between January 1995 and December 2001. Data represent single weekly depth-integrated samples. Data courtesy of NIWA monitoring program.

Recent studies have demonstrated that in some areas ENSO-correlated changes in wind stress affect upwelling (Roy and Reason 2001; Susanto et al. 2001). Upwelling is a major source of 'new' nutrients in coastal systems (Zeldis 2004). Wind-driven upwelling is often enhanced when wind stress is parallel to the continental shelf (Zeldis et al. 2004). The Coriolis force resulting from the earth's rotation causes the surface current to deviate to the left of wind direction in the southern hemisphere, causing a phenomenon known as Ekman transport (Mann and Lazier 1991). When Ekman transport forces surface water away from the coast, it is replaced by deeper, nutrient-rich water that upwells. This injection of inorganic nutrients into the system by upwelling is vital for primary productivity (Sverdrup 1955; Mann and Lazier 1991). Diatoms, in particular, thrive in upwelling conditions that provide high concentrations of nitrate for rapid growth (Margalef 1978; Smetacek 1985; Mann 1993) (Chapter 3).

Nitrate is the key limiting nutrient to phytoplankton in Beatrix Bay (Chapter 3, Gibbs and Vant 1997). The major source of nitrate for Beatrix Bay is advection from Cook Strait (Gibbs et al. 1992, 2002; Dupra 2000), an area where upwelling of nutrient-rich water occurs (Bowman et al. 1983; Greig et al. 1988; Viner and Wilkinson 1988; Harris 1990; Murdoch et al. 1990). The continental shelf at the entrance to Pelorus Sound has a 330°-150° orientation (approximately NW-SE) (Fig. 5.2), such that upwelling-favourable conditions through Ekman transport movement of surface water should arise when wind stress is from the northwest. Conversely, wind stress from the southeast should drive downwelling conditions. The prevailing winds through Cook Strait are from the northwest and the southeast due to the funnelling effects of the North and South Island (Harris 1990; Reid 1996). While southwest wind stress tends to be stronger in New Zealand during El Niño phases (Brenstrum 1998), during El Niño summers wind stress tends to be more westerly in direction (with a greater northwest component) (Gordon 1986; Mullan 1996). During La Niña phases northeast wind stress increases (Brenstrum 1998). Wind stress tends to be more easterly (with a greater southeast component) during La Niña summers (Gordon 1986; Mullan 1996). It is hypothesised that ENSO-driven changes in wind stress could alter the extent of upwelling in southeastern Cook Strait. This would affect the nitrate levels being advected into Pelorus Sound, and ultimately Beatrix Bay (Fig. 4.7), impacting on the phytoplankton community dynamics.

The other mechanism by which ENSO could potentially affect diatom-dinoflagellate dynamics is through ENSO-driven rainfall anomalies altering stratification in Pelorus Sound. Salinity stratification due to freshwater inflow is considered the major vertical structuring influence in Pelorus Sound (Gibbs 1993; Sutton and Hadfield 1997; Proctor and Hadfield 1998). Prolonged stratification of the water column leads to the upper layer becoming deficient in nutrients as primary producers use them up (Margalef 1978). Dinoflagellates can outcompete diatoms in strongly stratified, nutrient-poor conditions because their motility allows them to exploit both the overlying euphotic zone and underlying nutrient-rich waters (Margalef 1978; Cullen 1982; Mann 1993).

The impact of ENSO through changes in upwelling and salinity stratification are not investigated here as mutually exclusive hypotheses. In any system there will be a large number of physical and biological factors interacting to affect productivity. This chapter examines the evidence for large-scale climate-induced forcing driving phytoplankton dynamics in Pelorus Sound.

5.2 METHODS

5.2.1 Southern Oscillation Index (SOI)

The Southern Oscillation Index was used to characterise ENSO. It is calculated as the standardised anomalies of the monthly mean sea-level pressure difference between Tahiti and Darwin. There are a number of different methods of calculating SOI. The data series was obtained from the Australian Bureau of Meteorology website[1] which uses the Troup method to calculate SOI as follows:

$$SOI = 10 \frac{[\text{Pdiff} - \text{Pdiffav}]}{\text{SD (Pdiff)}}$$

where

Pdiff = (average Tahiti Mean Sea Level Pressure (MSLP) for the month) – (average Darwin MSLP for the month)

Pdiffav = long term average of Pdiff for the month in question

SD(Pdiff) = long term standard deviation of Pdiff for the month in question.

[1] http://www.bom.gov.au/climate/glossary/soi.shtml

Although other indices of ENSO have been developed (e.g. MEI (see Roy and Reason 2001), SOI was used as it conforms with most preceding studies on ENSO.

5.2.2 Wind Stress

Wind direction and velocity data, recorded at the NIWA meteorological station on The Brothers Island in western Cook Strait (Fig. 5.2), were averaged for the last 10 minutes of each hour. Wind stress data were available from January 1979 to May 2003. The continental shelf of this region has an approximate 330° – 150° orientation (Fig. 5.2) so along shelf wind components were calculated by adding 30° to the raw bearings to align the u and v components of the velocity cross-shelf and along-shelf, respectively. Along-shelf wind stresses (τ_{as}) were calculated using (Sharples and Greig 1998):

$$\tau_{as} = c_d p_a \sqrt{(u^2_w + v^2_w)} v_w$$

where p_a = 1.3 kg m^{-3} is the air density, u_w and v_w are the components of the wind velocity in the cross-shelf and along-shelf directions respectively, and c_d, the surface drag coefficient, is related to the wind speed, w, by:

$$c_d = (0.75+0.067w)\times 10^{-3}$$

When displayed graphically, negative along-shelf wind stress values represent wind stress from 330°, and positive values represent wind stress from 150°.

5.2.3 Sea Surface Temperature (SST)

Advanced Very High Resolution sea surface temperature (SST) data were obtained from the NIWA SST Archive (Uddstrom and Oien 1999) and are from the site -40.90S, 174.19E at the entrance to Pelorus Sound (Fig. 5.2). Sea surface temperature data were available from January 1994 to April 2003.

5.2.4 Pelorus River Flow

Pelorus River flow data were collected from the NIWA standard stream gauge at Bryants (Fig. 5.2), and flow rate calculated as in McKerchar (2002). River flow data were available between January 1979 and December 2003.

5.2.5 Phytoplankton and Nutrient Sampling

Data for phytoplankton biomass and nutrient concentration are courtesy of the NIWA monitoring program in Beatrix Bay. Data were available from December 1994 to January 2002. Methods of sampling and analyses are outlined in Chapter 2. Sampling consisted of single weekly samples. Samples were taken from the site WB (Fig. 2.1).

5.2.6 Data Manipulation and Analysis

All data were converted to monthly averages so they would conform with each other for statistical analysis. Because wind stress, sea surface temperature, Pelorus River flow, phytoplankton biomass, and nutrient concentration have seasonal patterns, these variables were converted to monthly anomalies. This eliminated any seasonal patterns from the data so that long-term, interannual variability could be examined. An anomaly is defined as the difference between the value of a variable for a given month and the average value for that month over the entire time series (as in Behrenfield et al. 2001). Three-monthly running means (as recommended by Trenberth 1976) and twelve-monthly running means were used to reduce noise at the monthly time-scale.

5.2.7 Canonical Correspondence Analysis

Canonical Correspondence Analysis (CCA) was used to investigate the relationship between seven environmental variables and taxa abundance. The environmental variables were: Southern Oscillation Index, sea surface temperature anomaly, along-shelf wind stress anomaly, ciliate grazer biomass anomaly, Pelorus River flow anomaly, nitrate concentration anomaly, and total dissolved inorganic nitrogen concentration anomaly. Taxa included in the analysis comprised at least 0.5% of total biomass and were present in at least 10% of samples. This resulted in an analysis of 25 taxa that formed 94.35% of overall phytoplankton biomass in Beatrix Bay. Taxa included in the analysis are listed in Appendix 5. Both environmental and taxa variables were smoothed using a 12-month running average. Canonical Correspondence Analysis was performed on Canoco v4.0.

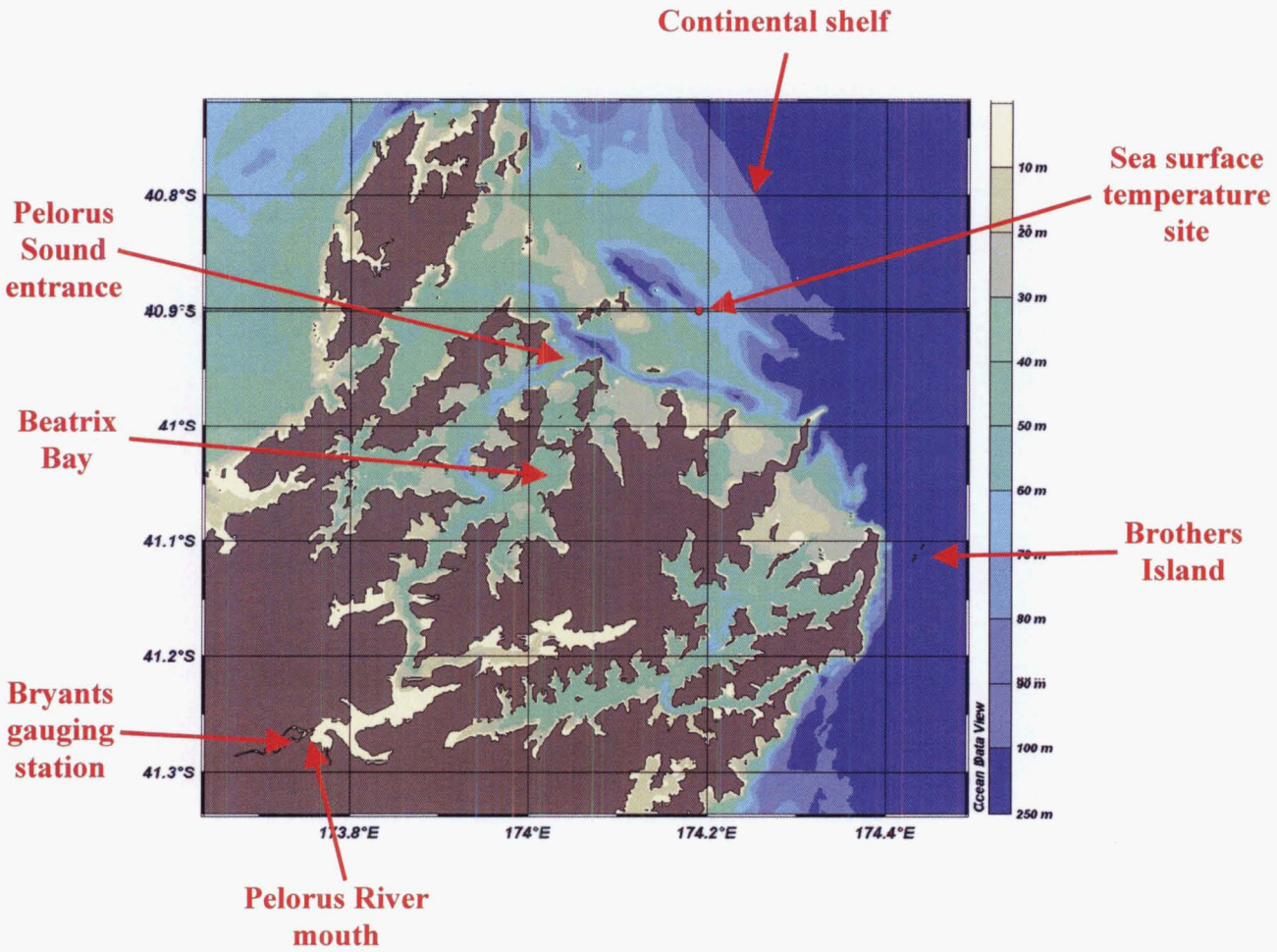


Figure 5.2. Map of Marlborough Sounds region showing the Cook Strait continental shelf (oriented at an angle of 330°), the site where sea surface temperature data were recorded (-40.90S, 174.19E), Brothers Island (site of NIWA meteorological station), and Bryants Pelorus River gauging station. Depth contours are indicated by colour bar on the right.

5.3 RESULTS

5.3.1 Phytoplankton Community

There appeared to be an inverse relationship between diatom biomass anomaly and dinoflagellate biomass anomaly throughout much of the time series, although this was not statistically significant at the 3-month smoothing level (Fig. 5.3a). This inverse relationship was particularly evident during the summer months in some years. During the summers of 1996-97 and 1997-98 diatom biomass was relatively high while dinoflagellate biomass was relatively low. During the summers of 1995-96, 1998-99 and 1999-2000 dinoflagellate biomass was relatively high while diatom biomass was relatively low. When the anomaly is smoothed further using a 12-month running average, it is apparent that in 1995 and from mid-1996 to mid-1998 diatoms were relatively successful while from mid-1998 to mid-2000 dinoflagellates were relatively successful (Fig. 5.3b).

5.3.2 Environmental variables

Strong El Niño phases (e.g. 1982, 1986, 1997-8) were associated with wind stress anomaly from 330° (approximately northwest) (Fig. 5.4 a, b). Strong La Niña phases (e.g. 1989, 1998-2000) were associated with wind stress anomaly from 150° (approximately southeast). During neutral phases of ENSO, wind stress anomaly could be strongly northwest (e.g. 1980), strongly southeast (e.g. 1985) or neutral (e.g. 1983).

The relationship between ENSO and along-shelf wind stress in Cook Strait was much stronger during summer than any other season (Table 5.1). The relationship was particularly strong when only ENSO phases of a magnitude greater than 10 were considered.

Along-shelf wind stress anomaly from the northwest was generally associated with colder sea surface temperature throughout the time series (Figure 5.5a, b). However, along-shelf wind stress anomaly from the southeast was associated with relatively cold sea surface temperature at some times (e.g. 1997), and warm sea surface temperature at other times (e.g. 2001).

The relationship between along-shelf wind stress anomaly and sea surface temperature anomaly in Cook Strait was much stronger during the summer months, particularly when the relationship was only considered during ENSO phases of magnitude greater than 10 (Table 5.2).

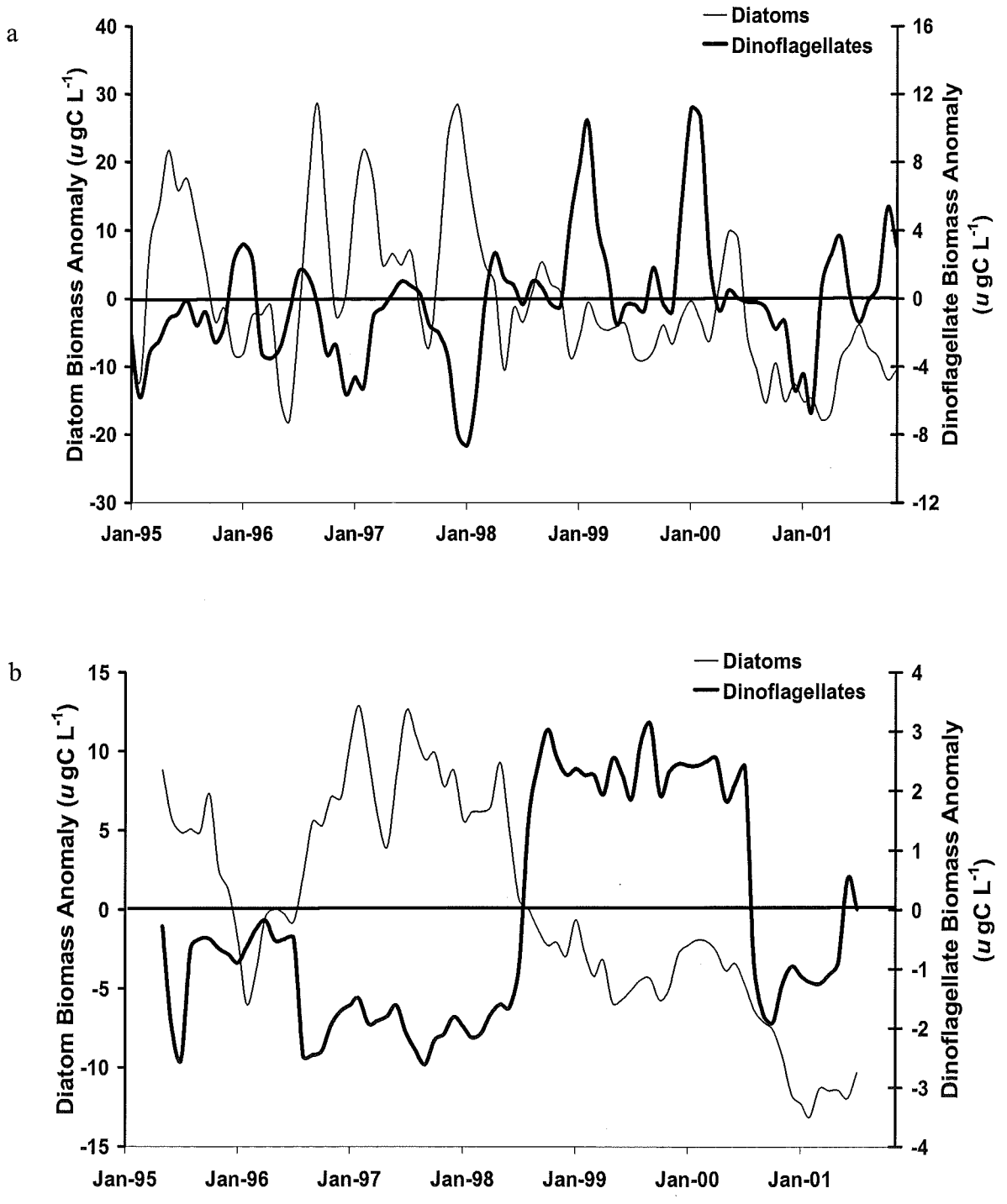


Figure 5.3. Diatom biomass anomaly and dinoflagellate biomass anomaly in Beatrix Bay between 1994 and 2002. a) Data smoothed using a 3-month running average. Spearmans rank order correlation, P=NS, r=-0.20. b) Data smoothed using a 12-month running average. Spearmans rank order correlation, P<0.000005, r=-0.53.

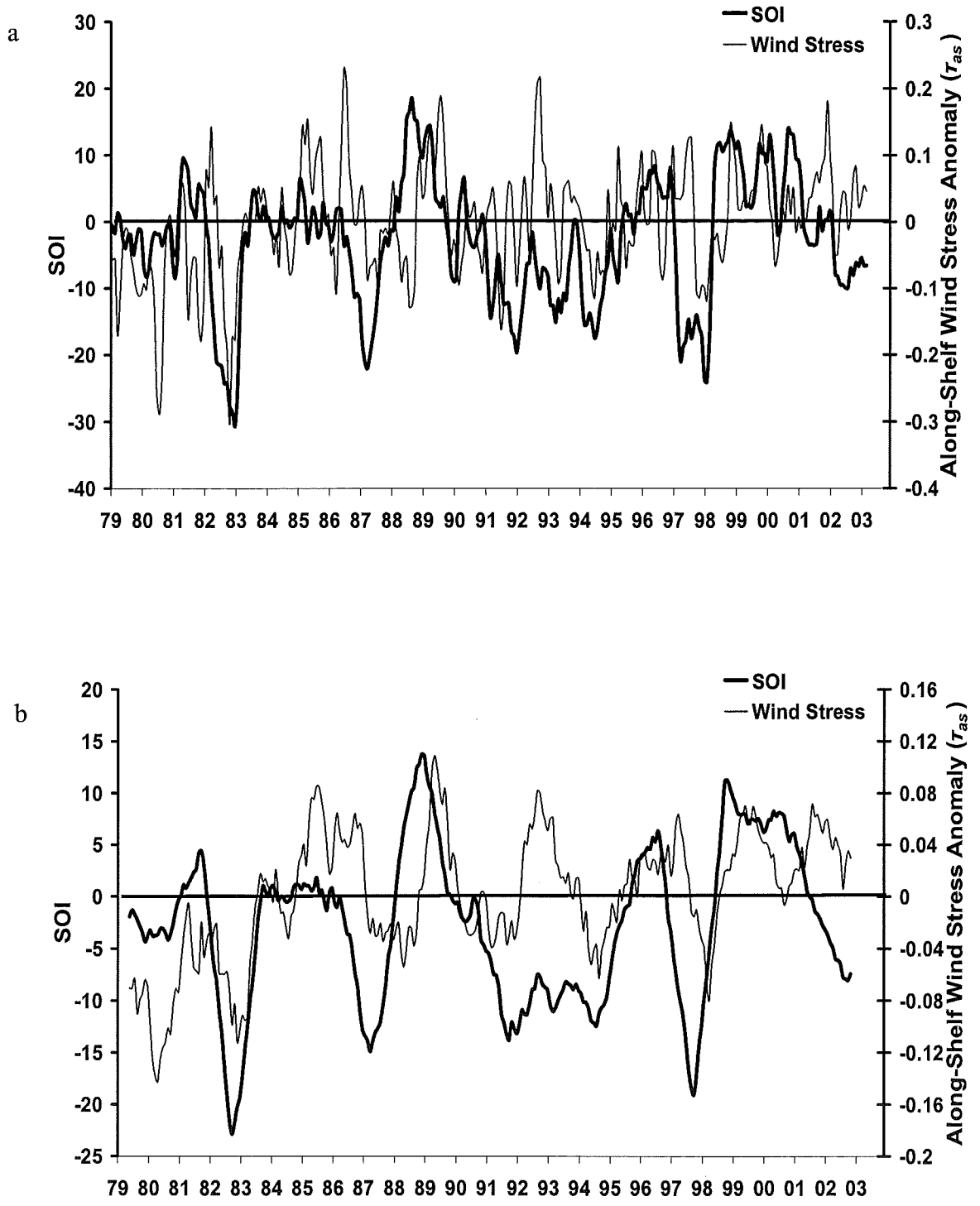


Figure 5.4. Southern Oscillation Index (SOI) and along-shelf wind stress anomaly at Brothers Island between 1979 and 2003. Negative along-shelf wind stress anomaly values indicate wind stress from 330°, positive values indicate wind stress from 150°. a) Data smoothed using 3-month running average. Spearmans rank order correlation, $P<0.0005$, $r=0.20$. b) Data smoothed using 12-month running average. Spearmans rank order correlation, $P<0.000001$, $r=0.32$.

Table 5.1. Seasonal relationship between SOI and along-shelf wind stress anomaly in Cook Strait during two ENSO conditions: all ENSO and ENSO conditions of a magnitude greater than 10. Spearmans rank order correlation r value displayed. * indicates statistically significant result (P<0.05).

Season	All ENSO	ENSO magnitude > 10
Summer	0.33 (*)	0.74 (*)
Autumn	0.11	0.42 (*)
Winter	-0.03	-0.20
Spring	-0.01	0.09

Table 5.2. Seasonal relationship between sea surface temperature anomaly and along-shelf wind stress anomaly in Cook Strait during two ENSO conditions: all ENSO and ENSO conditions of a magnitude greater than 10. Spearmans rank order correlation r value displayed. * indicates statistically significant result (P<0.05).

Season	All ENSO	ENSO magnitude > 10
Summer	0.47 (*)	0.82 (*)
Autumn	0.32	0.58
Winter	-0.20	-0.48
Spring	0.21	-0.08

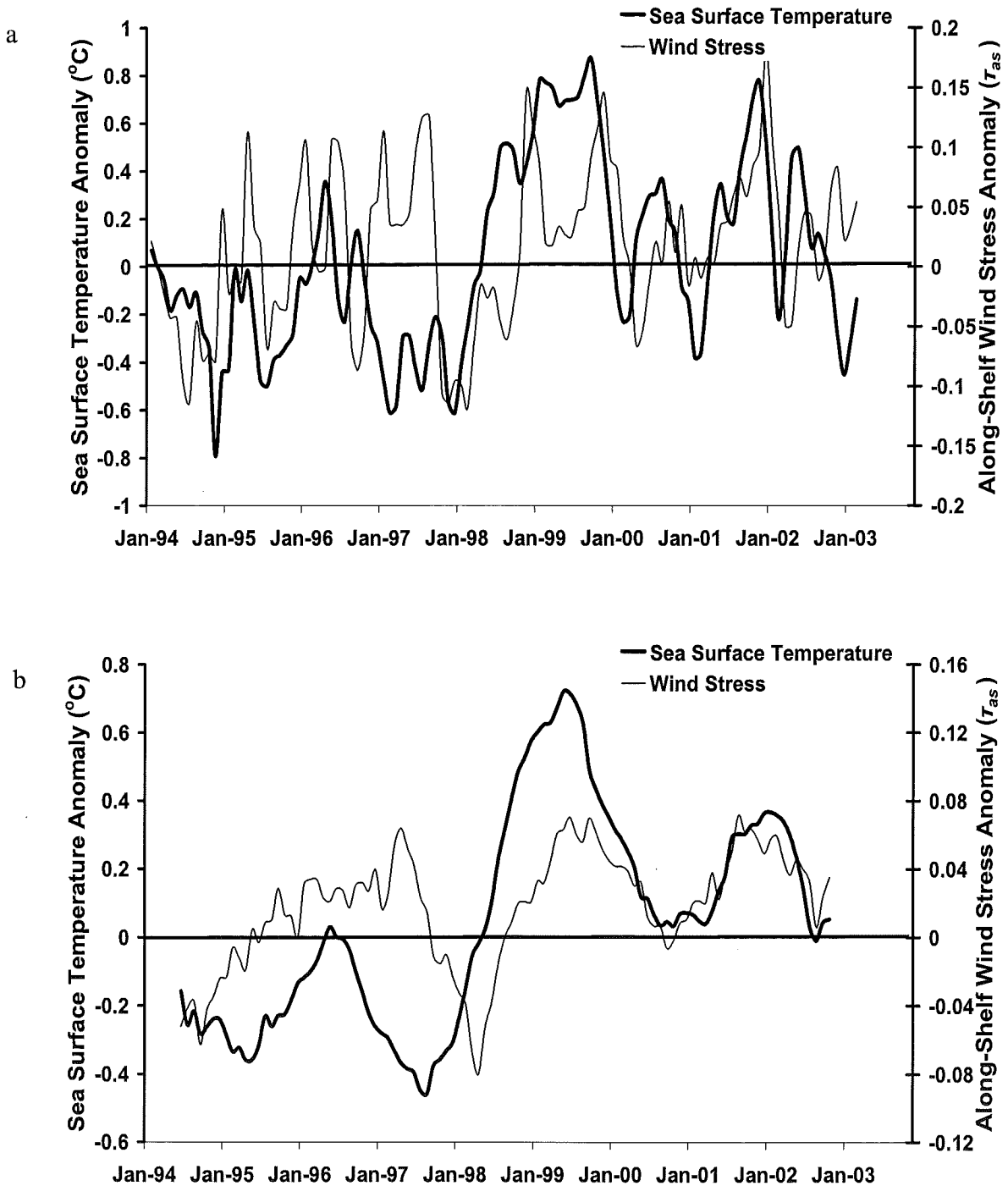


Figure 5.5. Sea surface temperature anomaly at the entrance to Pelorus Sound (-40.90S, 174.19E) and along-shelf wind stress anomaly at Brothers Island between 1994 and 2003. Negative along-shelf wind stress anomaly values indicate wind stress from 330°, positive values indicate wind stress from 150°. a) Data smoothed using 3-month running average. Spearmans rank order correlation, $P<0.02$, $r=0.22$. b) Data smoothed using 12-month running average. Spearmans rank order correlation, $P<0.000001$, $r=0.54$.

Colder sea surface temperatures are an indication of upwelling on the continental shelf. Most of the nitrate in Beatrix Bay is advected into the bay from Cook Strait via the main channel (Gibbs et al. 1992, 2002; Dupra 2000) (Fig. 4.7). However there was no relationship between sea surface temperature anomaly in Cook Strait and *in situ* nitrate or total dissolved inorganic nitrogen concentrations in Beatrix Bay (Fig. 5.6a, b). These results will be discussed later.

ENSO does not appear to drive long-term changes in Pelorus River flow (Fig. 5.7a, b). Low river flow occurred during both El Niño phases (e.g. 1982, 1997) and La Niña phases (e.g. 2000). Similarly, high river flow occurred during both El Niño phases (e.g. 1987) and La Niña phases (e.g. 1998). There does not appear to be a seasonal bias in the SOI-Pelorus River flow relationship (Table 5.3). The relationship is not statistically significant during any season.

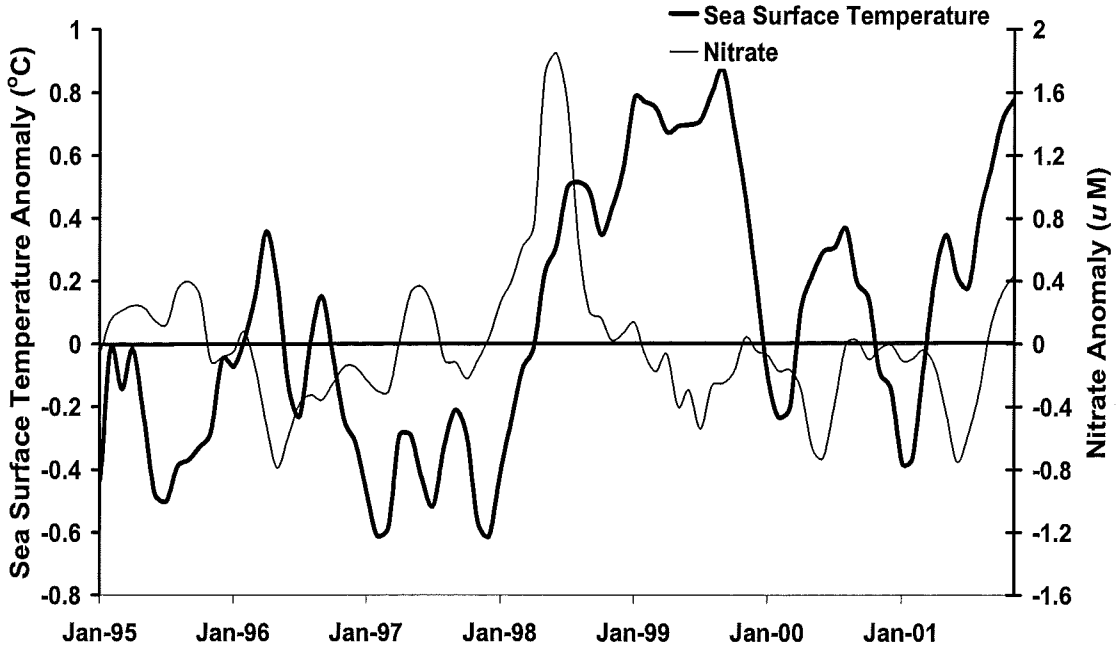
Table 5.3. Seasonal relationship between SOI and Pelorus River flow anomaly during two ENSO conditions: all ENSO and ENSO conditions of a magnitude greater than 10. Spearmans rank order correlation r value displayed. * indicates statistically significant result ($P<0.05$).

Season	All ENSO	ENSO magnitude > 10
Summer	0.03	0.21
Autumn	0.14	0.22
Winter	0.10	0.10
Spring	0.18	0.21

5.3.3 Environmental variables and phytoplankton community

Colder than normal sea surface temperatures tended to be associated with higher relative biomass of diatoms, while warmer sea surface temperatures were associated with lower diatom biomass throughout much of the time series (Fig. 5.8a, b). Conversely, higher sea surface temperatures were associated with higher relative dinoflagellate biomass and lower sea surface temperatures with lower dinoflagellate biomass throughout the time series (Fig. 5.9a, b).

a



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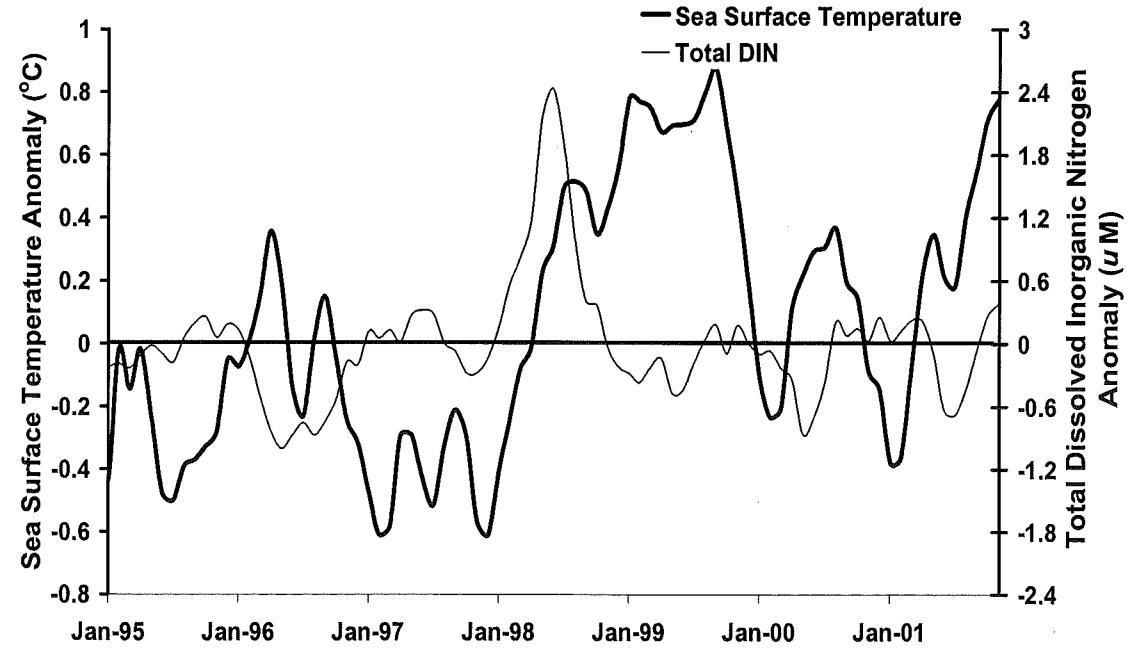

Figure 5.6. a) Sea surface temperature anomaly in Cook Strait and nitrate concentration anomaly in Beatrix Bay between 1994 and 2003. Data smoothed using 3-month running average. Spearmans rank order correlation, P=non-significant, r=-0.11. b) Sea surface temperature anomaly in Cook Strait and total dissolved inorganic nitrogen (DIN) concentration in Beatrix Bay between 1994 and 2003. Data smoothed using 3-month running average. Spearmans rank order correlation, P=non-significant, r=-0.08.

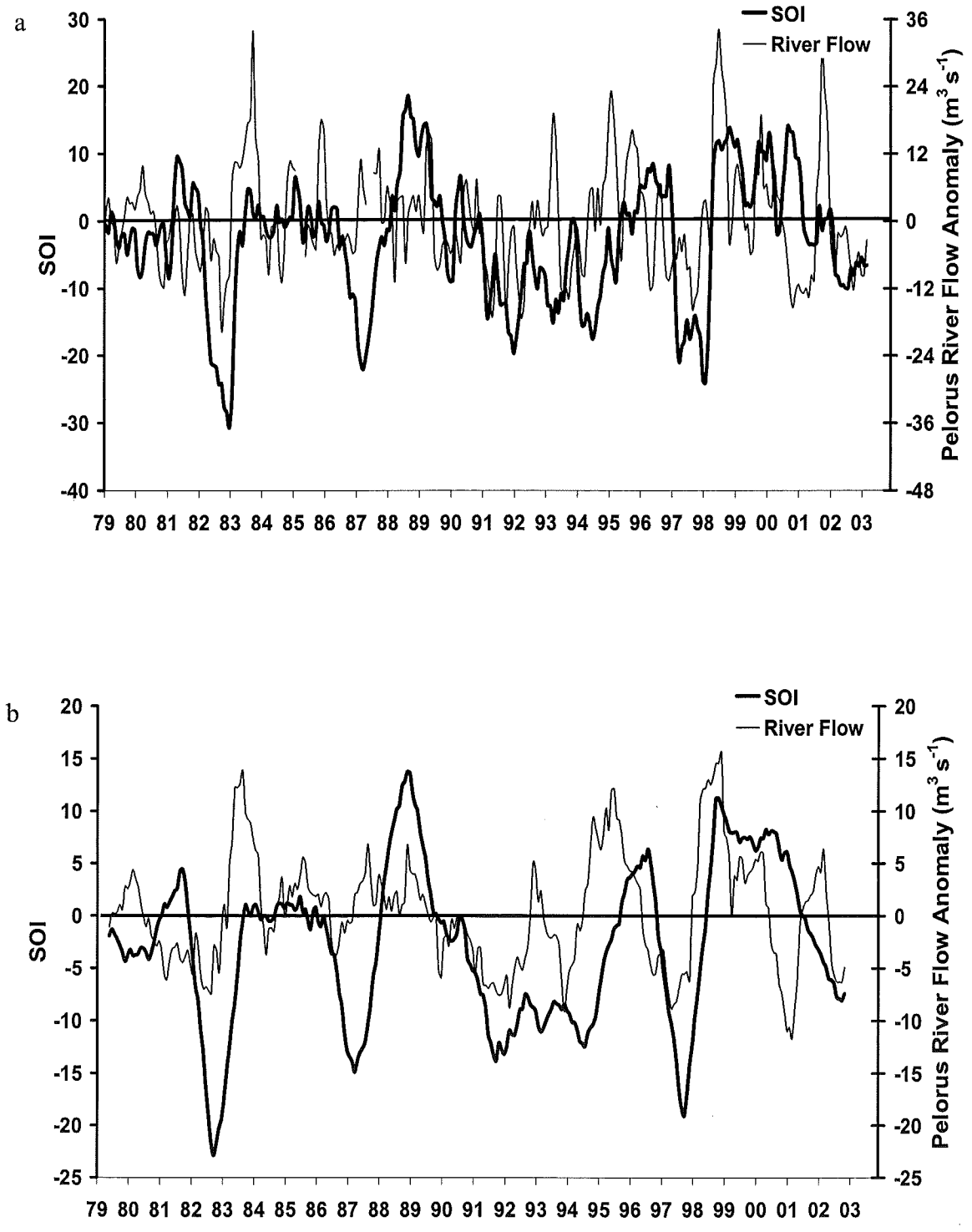

Figure 5.7. Southern Oscillation Index (SOI) and Pelorus River flow anomaly between 1979 and 2003. a) Data smoothed using 3-month running average. Spearmans rank order correlation, $P<0.03$, $r=0.22$. b) Data smoothed using 12-month running average. Spearmans rank order correlation, $P<0.02$, $r=0.24$.

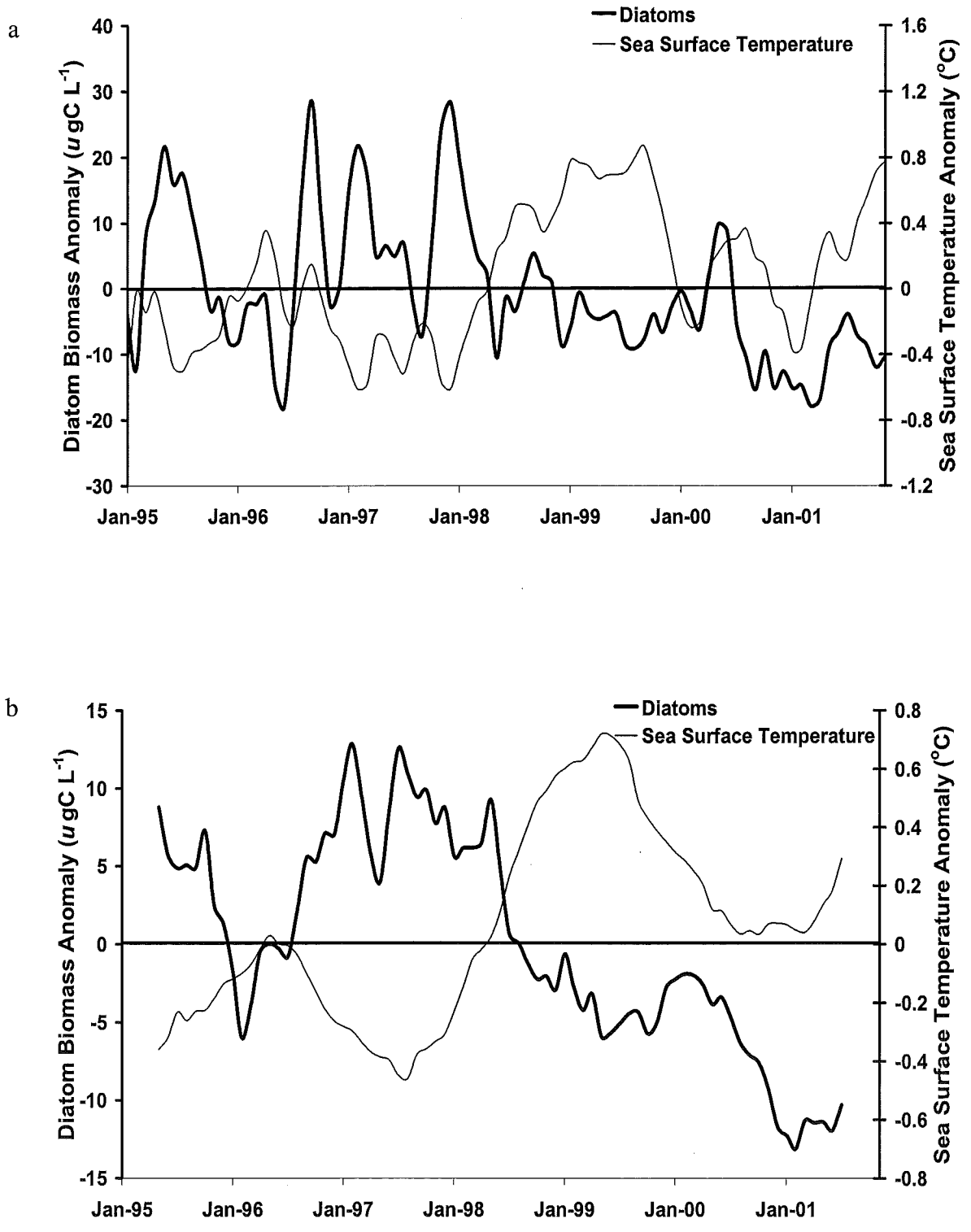

Figure 5.8. Diatom biomass anomaly in Beatrix Bay and sea surface temperature anomaly in Cook Strait between 1994 and 2002. a) Data smoothed using 3-month running average. Spearmans rank order correlation, $P<0.00005$, $r=-0.46$. b) Data smoothed using 12-month running average. Spearmans rank order correlation, $P<0.000001$, $r=-0.71$.

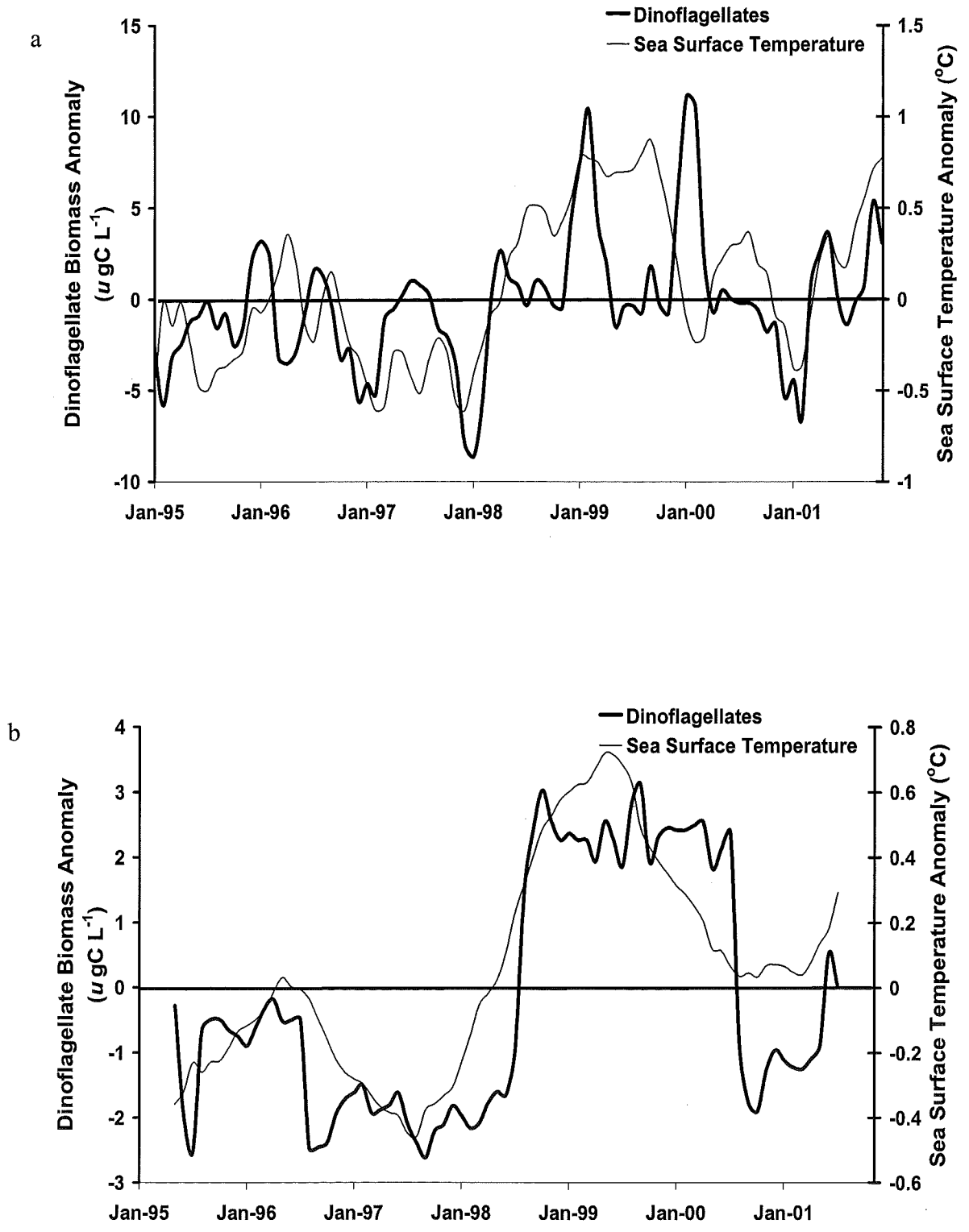

Figure 5.9. Dinoflagellate biomass anomaly in Beatrix Bay and sea surface temperature anomaly in Cook Strait between 1994 and 2002. a) Data smoothed using 3-month running average. Spearmans rank order correlation, $P<0.00005$, $r=0.45$. b) Data smoothed using 12-month running average. Spearmans rank order correlation, $P<0.000001$, $r=0.78$.

There was no clear relationship between relative diatom biomass and relative Pelorus River flow (Fig. 5.10a, b). This relationship is not statistically significant at either the 3-month or the 12-month smoothing level. Periods of lower than normal river flow tended to be associated with lower relative dinoflagellate abundance (Fig. 5.11a, b). However, periods of relatively high river flow could be associated with high dinoflagellate biomass (e.g. January 1999, late 2001) or low dinoflagellate biomass (e.g. January 1995, mid 1998) (Fig. 5.11a).

The seven environmental variables used in the canonical correspondence analysis explained 73.70% of variation in taxa abundance (Table 5.4). Each of the environmental variables explained a significant amount of variation in taxa abundance (Monte-Carlo test, $p<0.05$). Sea surface temperature anomaly and Southern Oscillation Index were the best-performed environmental variables explaining 27.52% and 27.22% of taxa abundance (Table 5.5). *In situ* nitrate concentration anomaly was the poorest performed environmental variable, explaining just 7.03% of variation in species abundance. The results are displayed graphically in Figure 5.12, with environmental variables furthest from the origin the most powerful in explaining taxa variability and variables close together being closely positively correlated.

Table 5.4. Summary statistics for Canonical Correspondence Analysis on environmental variables and abundance of 25 key phytoplankton taxa.

Axes	**1**	**2**	**3**	**4**	**Total Inertia**
Eigenvalues	0.109	0.079	0.021	0.016	0.327
Species-environment correlations	0.955	0.925	0.843	0.832	
Cumulative percentage variance of:					
species data	33.3	57.6	63.9	68.8	
species-environment relationships	45.3	78.2	86.8	93.5	
Sum of all unconstrained eigenvalues					0.327
Sum of all canonical eigenvalues					0.241

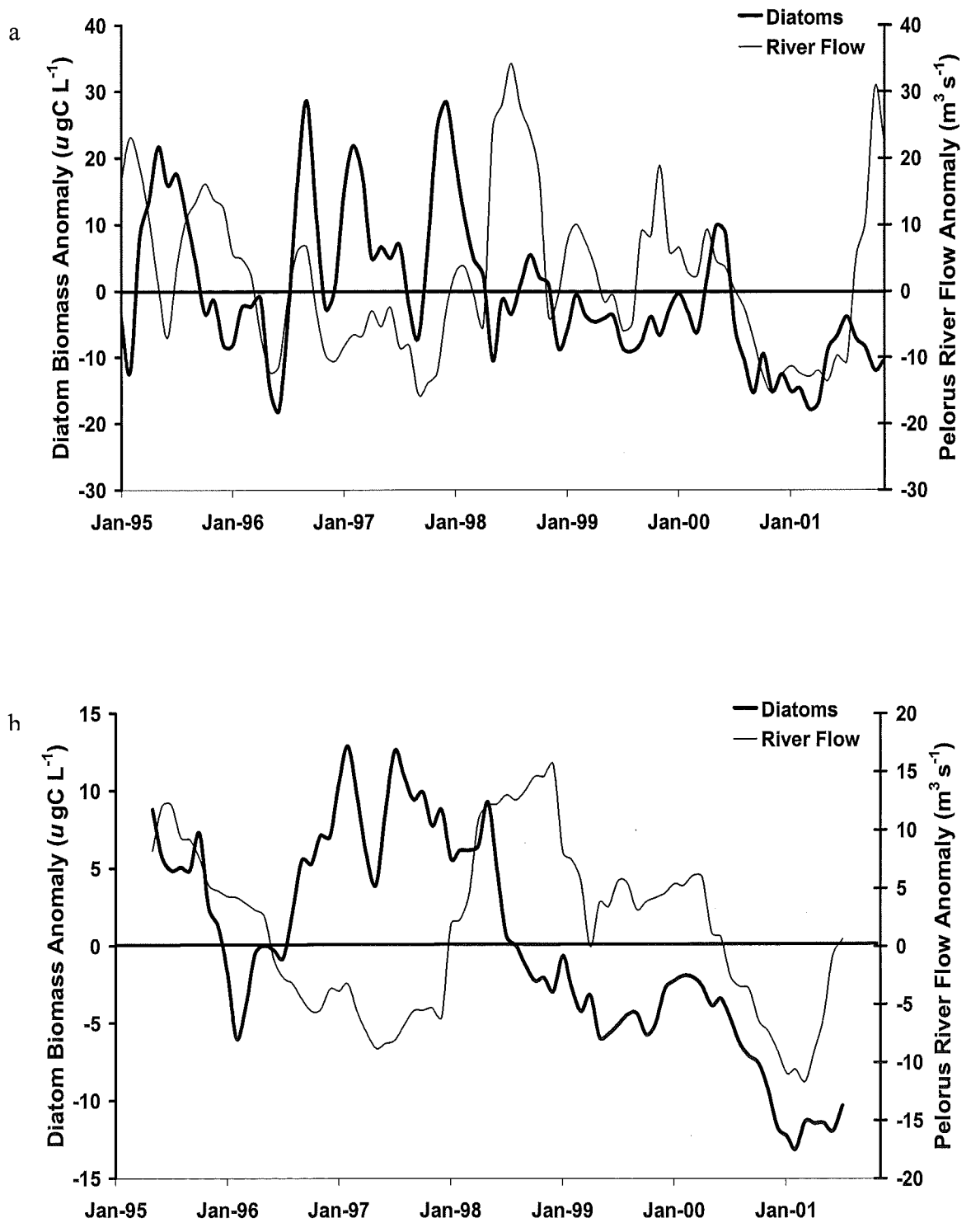

Figure 5.10. Diatom biomass anomaly in Beatrix Bay and Pelorus River flow anomaly between 1994 and 2002. a) Data smoothed using 3-month running average. Spearmans rank order correlation, P=NS, r=0.14. b) Data smoothed using 12-month running average. Spearmans rank order correlation, P=NS, r=0.07.

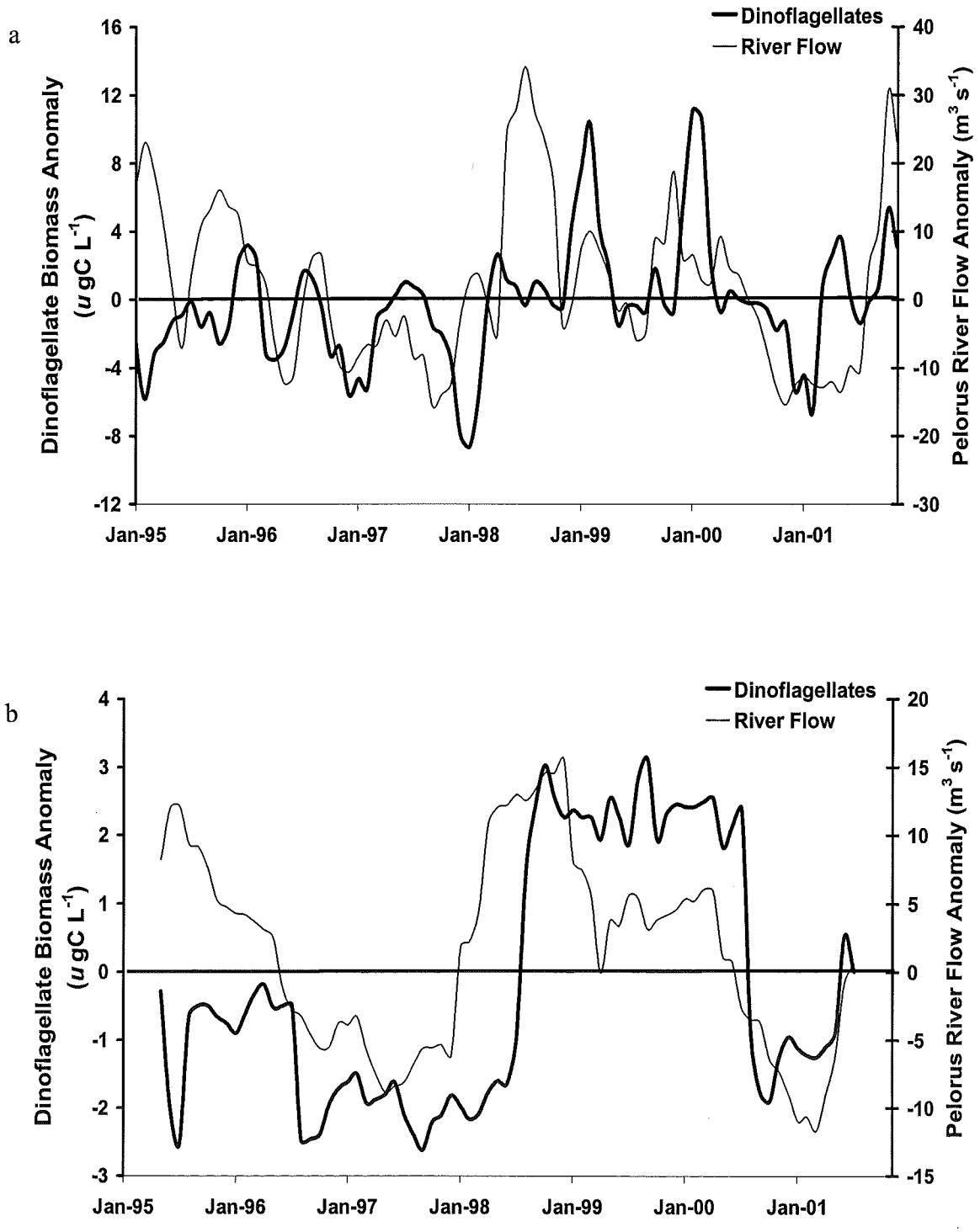

Figure 5.11. Dinoflagellate biomass anomaly in Beatrix Bay and Pelorus River flow anomaly between 1994 and 2002. a) Data smoothed using 3-month running average. Spearmans rank order correlation, $P<0.005$, $r=0.32$. b) Data smoothed using 12-month running average. Spearmans rank order correlation, $P<0.000005$, $r=0.50$.

Table 5.5. Eigenvalue coefficients from Canonical Correspondence Analysis of environmental variables and taxa abundance.

Environmental Variables	Eigenvalues	% Variation Explained
Sea Surface Temperature	0.090	27.52
Southern Oscillation Index	0.089	27.22
Ciliate Grazers	0.055	16.82
Wind Stress	0.037	11.31
Total DIN	0.029	8.87
River Flow	0.026	7.95
Nitrate	0.023	7.03
Cumulative Total	**0.241**	**73.70**

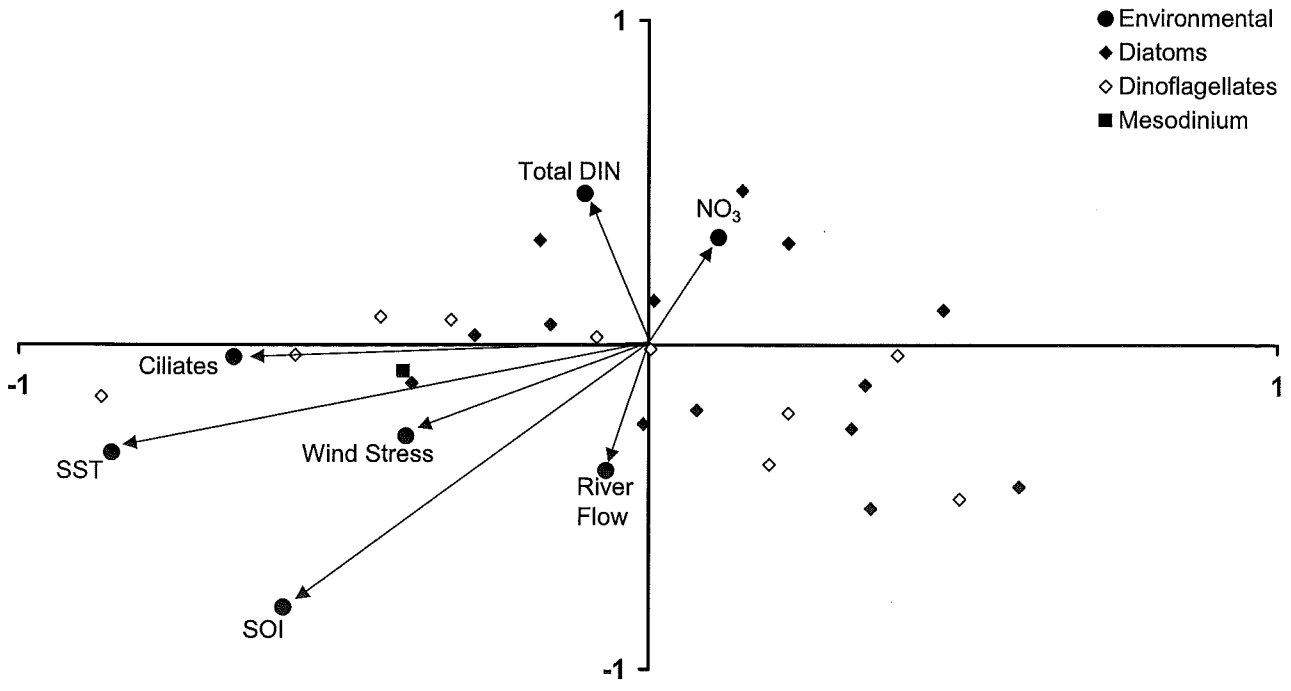

Figure 5.12. Results of Canonical Correspondence Analysis on environmental variables and key taxa. Environmental variables furthest from the origin explain the most variation in taxa abundance. Variables close together are positively correlated.

5.4 DISCUSSION

5.4.1 ENSO and wind-driven upwelling

A flow diagram summarising the evidence for ENSO driving interannual phytoplankton variability through anomalies in wind-driven upwelling is shown below (Fig. 5.13). Between 1979 and 2003 SOI was correlated with along-shelf wind stress in Cook Strait during summer (Table 5.1). Seasonal variation in the impact of SOI on New Zealand weather has been documented elsewhere. Gordon (1986) and Mullan (1995) reported that wind flow anomaly is westerly for a negative SOI during summer months in New Zealand, as opposed to southwest during other seasons. Wind low anomaly is easterly for a positive SOI during summer months, as opposed to northeast during other seasons (Gordon 1986; Mullan 1996). Westerly wind stress would have a greater component in the northwest direction required to drive upwelling in Cook Strait, and likewise easterly wind stress would have a greater component in the downwelling-favourable southeasterly direction. The relationship between SOI and along-shelf wind stress in summer was especially strong when the data were stratified to include only ENSO events of a magnitude greater than ten. Mullan (1996) also found that when SOI was near zero there was a great deal of scatter in wind direction and large positive or negative wind stress anomalies could occur.

Although the relationship between along-shelf wind stress and sea surface temperature in Cook Strait was statistically significant, this relationship appeared to break down during some periods of southeast wind stress (Fig. 5.5a). Despite southeast wind stress being favourable for downwelling and therefore warmer sea surface temperatures, there were periods of relatively cold sea surface temperature during southeast wind stress. It is interesting that the relationship between along-shelf wind stress and sea surface temperature was much stronger during summer than any other season, and especially strong during ENSO phases of a magnitude greater than ten (Table 5.2). Because along-shelf wind stress measures the component of wind in the northwest or southeast direction, this result could reflect the fact that during summer (and during periods when ENSO magnitude is greater than ten), wind stress tends to be more westerly-easterly. These wind directions are more favourable for upwelling and downwelling respectively along the Cook Strait continental shelf. During other times wind stress may be in a direction not as favourable for upwelling (or downwelling), but might still have a significant component in the northwest (or southeast) direction.

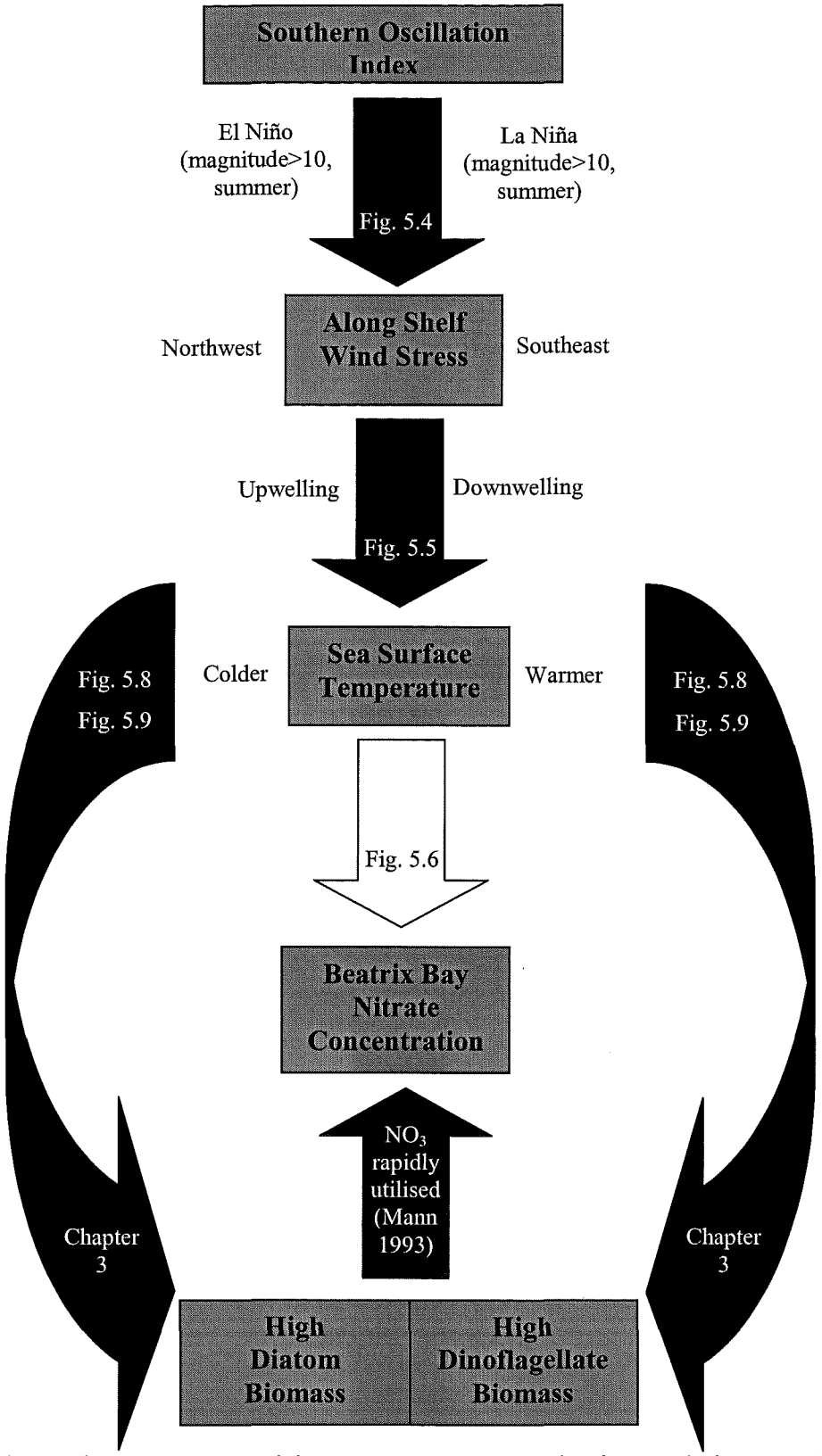

Figure 5.13. Flow diagram summarising a proposed mechanism relating ENSO to phytoplankton dynamics in Beatrix Bay through nutrient supply, and outlining the evidence for this mechanism. Black arrows indicate connections supported by evidence in this thesis or in the literature. White arrow indicates connection not supported by evidence in this thesis. The scenario on the left-hand side of each box or arrow (beginning at the top with an El Niño phase), leads to a high biomass of diatoms. The scenario on the right hand side of each box or arrow (beginning at the top with a La Niña phase), leads to a high biomass of dinoflagellates.

Other studies have shown wind driven upwelling to occur along the Cook Strait continental shelf. Murdoch et al. (1990) used vertical temperature-salinity profiles to demonstrate upwelling at the southeast end of Cook Strait during a period of sustained northerly winds. Bowman et al. (1983) used similar methods to conclude that upwelling occurs in Cook Strait under northwest winds, and cited the area outside Pelorus Sound as an area where upwelling occurs. My study lacks sufficient oceanographic data in Cook Strait to determine if changes in sea surface temperature are due to upwelling. Long-term Cook Strait nitrate monitoring has only recently been implemented and no long-term water column data exist. However, the relationship shown here between SOI, wind stress, and sea surface temperature, coupled with the existing knowledge of the area strongly suggests a link between SOI and upwelling in Cook Strait during summer.

Few other long-term studies have linked SOI to upwelling over multiple ENSO cycles. Susanto et al. (2001) discovered a strong relationship between ENSO condition and upwelling off the coasts of Java and Sumatra between 1981 and 1999. Tanasichuk (2002) reported increased upwelling along the southwest Vancouver Island coast during 1992 and 1998 El Niño years. Most studies in the literature follow a single transition from an El Niño to La Niña phase. Inferring information from short-term studies such as these can be dangerous as correlations often hold for a few years then fail (Mann 1993).

The lack of a relationship between sea surface temperature (presented here as an indicator of upwelling) and *in situ* nitrate or total dissolved inorganic nitrogen concentrations in Beatrix Bay (Fig. 5.6a, b) is not necessarily surprising. Nutrient sampling was undertaken in the upper 15 m of the water column, where light levels in Beatrix Bay are sufficient to support net phytoplankton growth (Gibbs and Vant 1997). Nitrogen-limited phytoplankton would be expected to quickly use up any newly available nitrate in the upper water column. Therefore, upwelling might not translate directly into increased nitrate levels in the upper water column if nitrate-depleted phytoplankton rapidly use the nutrient up (Mann 1993).

The correlations between sea surface temperature in Cook Strait and diatom and dinoflagellate abundance in Beatrix Bay (Fig. 5.8, 5.9) support the use of sea surface temperature as an indicator of upwelling and downwelling. Anomalously cold sea surface temperature (assumed to represent upwelling) in Cook Strait was accompanied by a relatively high biomass of diatoms and low biomass of dinoflagellates in Beatrix Bay. Warmer sea

surface temperatures (assumed to represent downwelling) were correlated to increased dinoflagellate and decreased diatom biomass. Other studies have shown diatoms to be successful under upwelling conditions and dinoflagellates to outcompete diatoms when nutrients are limiting. Parsons et al. (1978) found that diatoms were able to dominate in upwelling simulated conditions due to their ability to multiply rapidly when conditions are favourable. When inorganic nitrogen was low, flagellates were favoured. Pitcher et al. (1993) used microcosms to simulate upwelling conditions and discovered that diatoms dominated during the phytoplankton biomass peak. Chang et al. (2003) reported that upwelling events in the Hauraki Gulf in north-eastern New Zealand were associated with increased diatom biomass. When the water column was strongly stratified and nutrient limited, dinoflagellates dominated phytoplankton biomass.

The performance of sea surface temperature and SOI in explaining the most variation in phytoplankton taxa abundance in the CCA strengthens the argument that SOI affects Beatrix Bay phytoplankton through upwelling. It is interesting to note that nitrate was one of the worst performed environmental variables. This is despite the fact that nitrate availability along with light availability is postulated in numerous studies (e.g. Gibbs et al. 1992, 2002; Gibbs and Vant 1997; Ross et al. 1998a; Gall et al. 2000) to be the primary limiting factor of phytoplankton production in Pelorus Sound. The explanation presented earlier that newly injected nitrate would go undetected when rapidly used up by phytoplankton appears to apply here.

5.4.2 ENSO and Pelorus River flow

A flow diagram summarising the evidence for ENSO driving interannual phytoplankton variability through anomalies in Pelorus River flow and stratification is shown below (Fig. 5.14). Pelorus River flow was used in this study as a proxy for salinity stratification in the absence of reliable, long-term stratification data. The Pelorus River, with a catchment of 880 km^2 and an average freshwater input of 43.0 m^3s^{-1}, is the single largest source of freshwater input into Pelorus Sound (Heath 1974). It is therefore logical that Pelorus River flow would be directly related to salinity stratification. Results from Chapter 3 demonstrated that the degree of salinity stratification in Beatrix Bay was related to prior Pelorus River flow (Table 3.1, Fig. 3.5a). Gibbs et al. (1991), Gibbs (1993), and Sutton and Hadfield (1997) all used Pelorus River flow as an indicator of overall freshwater input in previous studies on Pelorus Sound.

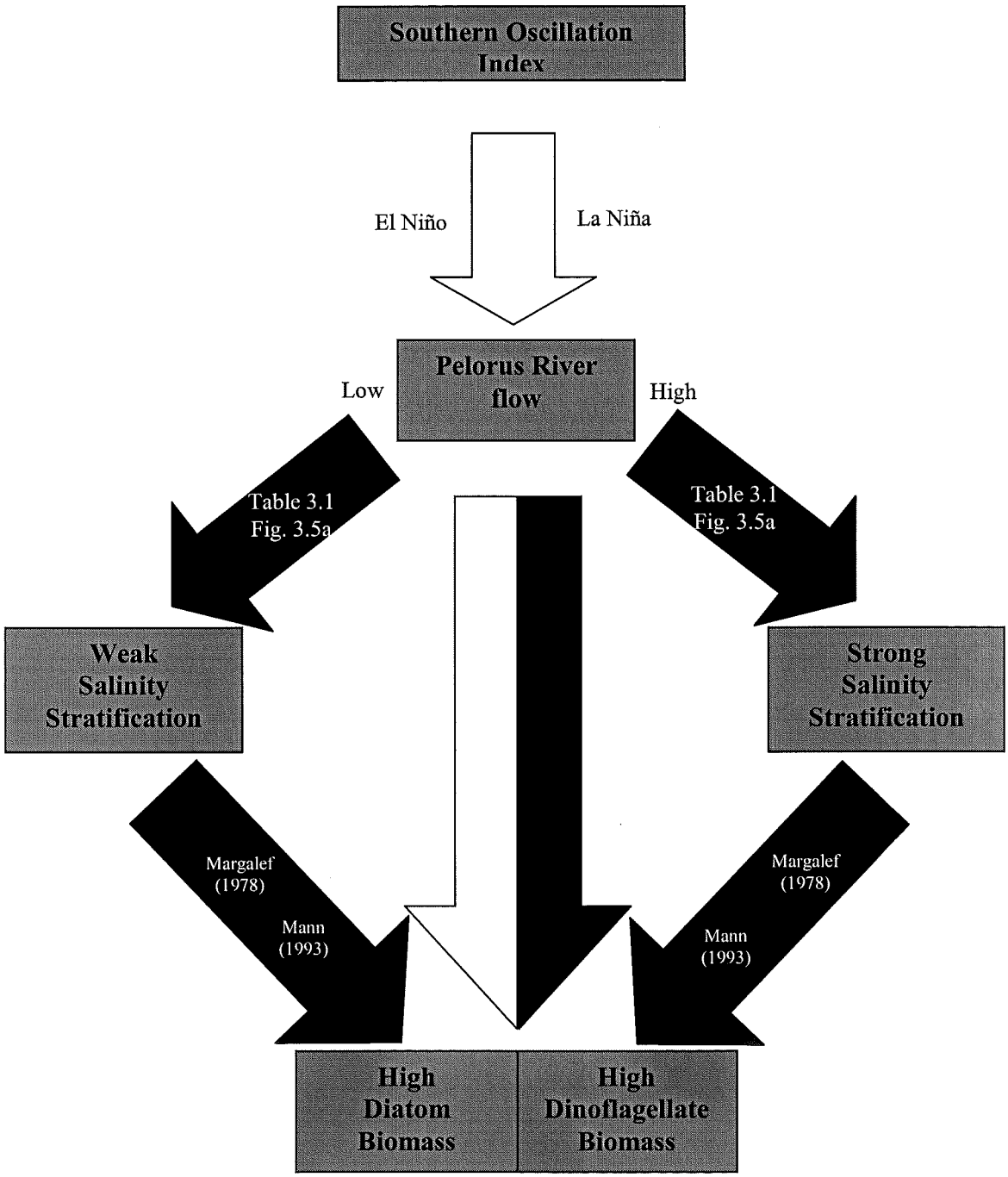

Figure 5.14. Flow diagram summarising a proposed mechanism relating ENSO to phytoplankton dynamics in Beatrix Bay through Pelorus River flow, and outlining the evidence for this mechanism. Black arrows indicate connections supported by evidence in this thesis or the literature. White arrows indicate connections not supported by evidence in this thesis. The scenario on the left-hand side of each box or arrow (beginning at the top with an El Niño phase), leads to a high biomass of diatoms. The scenario on the right hand side of each box or arrow (beginning at the top with a La Niña phase), leads to a high biomass of dinoflagellates.

The relationship between SOI and Pelorus River flow was weak, with both high and low river flow occurring during both El Niño and La Niña phases. There was no obvious seasonal variation in the SOI-Pelorus River flow relationship, even when only strong ENSO events were considered. Pelorus River flow was the 2nd worst performed variable in the CCA. These results are perhaps not surprising. Gordon (1986) claimed that rainfall throughout New Zealand was not well correlated with SOI. Mosley's (2000) analysis of hydrological data from 18 New Zealand rivers between 1948 and 1998 lead him to conclude that few rivers respond to El Niño and La Niña in opposite ways. McKerchar and Pearson (1994) found correlations between river flow and SOI were stronger in the North Island. The relationship between diatom biomass and Pelorus River flow was even weaker. Dinoflagellate abundance appeared better correlated to Pelorus River flow, and this may reflect a greater dependence of dinoflagellates on strongly stratified conditions to outcompete diatoms (Margalef 1978; Mann 1993).

5.4.3 Conclusion

This study provides evidence that long-term ENSO-correlated changes in upwelling along the Cook Strait continental shelf can impact phytoplankton dynamics in Beatrix Bay during summer. The relationship between ENSO, river-flow and phytoplankton dynamics was not as strong. This does not mean ruling it out as a factor influencing long-term phytoplankton dynamics in Beatrix Bay. There is a clear link between Pelorus River flow and salinity stratification (Table 3.1, Fig. 3.5a), and stratification of the water column is important in influencing nutrient and light availability to phytoplankton (Diehl 2002). However, there does not appear to be a strong, long-term relationship between ENSO, Pelorus River flow, and phytoplankton community dynamics in Beatrix Bay.

Summer is usually the time of highest dinoflagellate biomass in Beatrix Bay. It appears that during strong El Niño phases, increased wind-driven upwelling in Cook Strait can cause diatoms to persist during the summer and outcompete dinoflagellates. During strong La Niña phases in summer, dinoflagellates tend to prosper in Beatrix Bay.

CHAPTER 6
General Discussion

6.1 INTRODUCTION

The central objective of this thesis was to investigate the spatial and temporal processes that structure the phytoplankton community in a coastal ecosystem. Long-term monitoring of phytoplankton and nutrient levels provided invaluable background information into the structure of the phytoplankton community temporally and spatially. This information was complemented with more intensive sampling and enclosure experiments that manipulated nutrient concentration and light levels to test how these affected phytoplankton community dynamics.

6.2 WATER COLUMN STRUCTURE

Stratification is a prominent feature of coastal ecosystems worldwide for a number of reasons. Tidal velocities are often attenuated significantly within embayments, reducing the potential for mixing (Stevens 2003). The reduced fetch of sheltered embayments can dampen the influence of wind as a source of kinetic energy to mix the water column. Any riverine inputs are a source of low-density freshwater that promotes salinity-driven stratification of the water column.

Stratification is important in determining the supply of nutrients and light to phytoplankton (Diehl 2002). Phytoplankton trapped in the surface layer of a stratified water column are exposed to higher mean irradiance than the average irradiance throughout the water column, but are denied access to nutrients in bottom waters (Margalef 1978). These conditions favour motile dinoflagellates, which can vertically migrate to exploit both the overlying euphotic zone and underlying nutrient-rich waters (Margalef 1978; Cullen 1982; Mann 1993). Nutrient supply from bottom waters is enhanced in a well-mixed water column, but the average irradiance throughout the water column in which phytoplankton are mixed is reduced. The effects of nutrients and light on phytoplankton community composition were examined in this study and are discussed later.

Stratification also affects the rate of sinking losses of cells (Diehl 2002). Most phytoplankton species are denser than the surrounding medium and consequently tend to move downwards (Reynolds 1984). Sinking losses out of the euphotic zone can amount to >25% of the population per day, and can be important in influencing phytoplankton community composition (Diehl 2002). Motile dinoflagellates, which can maintain their position under weakly turbulent conditions using locomotion, are adapted to a stratified water column (Margalef 1978; Mann 1993). Non-motile diatoms will generally sink out of the water column under strongly stratified conditions, and depend on strong vertical turbulence to maintain their position in the water column (Margalef 1978; Mann 1993). Sinking rates are inversely correlated with growth rates in the majority of diatom taxa (Smayda 1970). Diatom sinking rates tend to increase under nutrient stress, and decrease when higher nutrient concentrations are encountered (Smayda 1970; Bienfang et al. 1982; Smetacek 1985). Diatom sinking rates differ between species and, therefore, may be a factor influencing phytoplankton community structure (Smayda 1970; Bienfang et al. 1982). For example, Harrison et al. (1986) experimentally showed that *Skeletonema* sp., which is very sensitive to silicate limitation, sank more quickly than *Chaetoceros* sp. in silicate-limited enclosures, but not in ammonium-limited enclosures.

Measurements of water column structure during this research confirmed the view of Proctor and Hadfield (1996, 1998) and Sutton and Hadfield (1997) that the Beatrix Bay water column is stratified, to some degree, most of the time (Fig. 3.3). The water column was thermally stratified during summer when irradiance levels were high (Table 3.1, Fig. 3.5b). During the rest of the year stratification was salinity-driven and related to the level of freshwater input from the Pelorus River (Table 3.1, Fig. 3.5a). The varying freshwater input generates constantly evolving water column stratification from autumn to spring (Stevens 2003).

6.3 NUTRIENTS

Although nutrients such as phosphate (Howarth 1988; Krom et al. 1991; Andersson et al. 1996) and silicate (Egge and Aksnes 1992; Fisher et al. 1992) have been found to limit phytoplankton growth in some marine waters, nitrogen is considered to be the primary nutrient limiting phytoplankton production in most coastal waters (Oviatt et al. 1995). The dominant form of nitrogen in the ocean is nitrate, and it is often this form that is taken up by phytoplankton (Lalli and Parsons 1993). Many species can also utilise recycled nitrogen in

the form of ammonium. Ambient nitrate levels in Beatrix Bay were low during the summer (below the detection limit of ~ 0.04 μM during January 2002) and high during winter (3.9 μM during July 2002) (Fig. 3.6a). Ambient ammonium fluctuated between 0.2 μM in summer and 0.4 μM in winter (Fig. 3.6b). There was a significant phytoplankton growth response to experimental nitrate addition during spring/summer, indicating nitrate-limitation of phytoplankton at this time. Previous studies in Beatrix Bay have also experimentally demonstrated nitrate limitation of phytoplankton growth during spring and summer (Gibbs and Vant 1997; Ogilvie et al. 2000). In contrast, phosphate and silicate were not found to be at limiting levels to phytoplankton during this study. Ambient phosphate concentrations were never below 0.1 μM (Fig. 3.6c), consistent with the findings of Gibbs and Vant (1997). Gibbs and Vant (1997) conducted phosphate enrichment experiments in cubitainers and did not find any significant phosphate-enhanced phytoplankton growth at any time of the year in Beatrix Bay. Egge and Aksnes (1992) experimentally demonstrated that silicate is limiting to diatom growth at concentrations below 2 μM. Silicate concentration was never below 3 μM during this study, and was frequently above 20 μM (Fig. 3.6d).

The nitrate-addition experiments of Chapter 3 demonstrated that the growth response to nitrate varied between phytoplankton taxa, and was based on taxonomic and morphological characteristics of the phytoplankton (Fig. 3.11, Fig. 3.13, Fig. 3.15). The largest growth response to nitrate addition occurred in small to medium sized, chain-forming diatom taxa such as *Chaetoceros* sp., *Pseudonitzschia* sp., *Skeletonema* sp., and *Thalassiosira* sp. (Table 3.15). These taxa dominated Beatrix Bay phytoplankton biomass between 1994 and 2002 (Fig. 3.17). Figure 6.1 shows the biomass of small to medium sized chain-forming diatom taxa and total phytoplankton biomass in Beatrix Bay from 1995 to 2002. It is evident that, apart from summer blooms (that consist primarily of dinoflagellates, Fig. 1.5), the total biomass in Beatrix Bay is dominated mostly by the biomass of these chain-forming diatom taxa throughout the year.

Dominance by chain-forming diatoms appears to be linked not only to nutrient concentration, but also to the frequency of nutrient supply. Pulsed nutrient supply has been experimentally shown to favour fast-growing phytoplankton species over slow-growing species (Sakshaug and Olsen 1986; Roelke et al. 1999). Fast-growing chain-forming diatoms are characteristic dominants of phytoplankton communities with frequent inputs of new nitrogen (Malone 1980). In Beatrix Bay, the dominance of diatoms throughout most of the year is likely to be

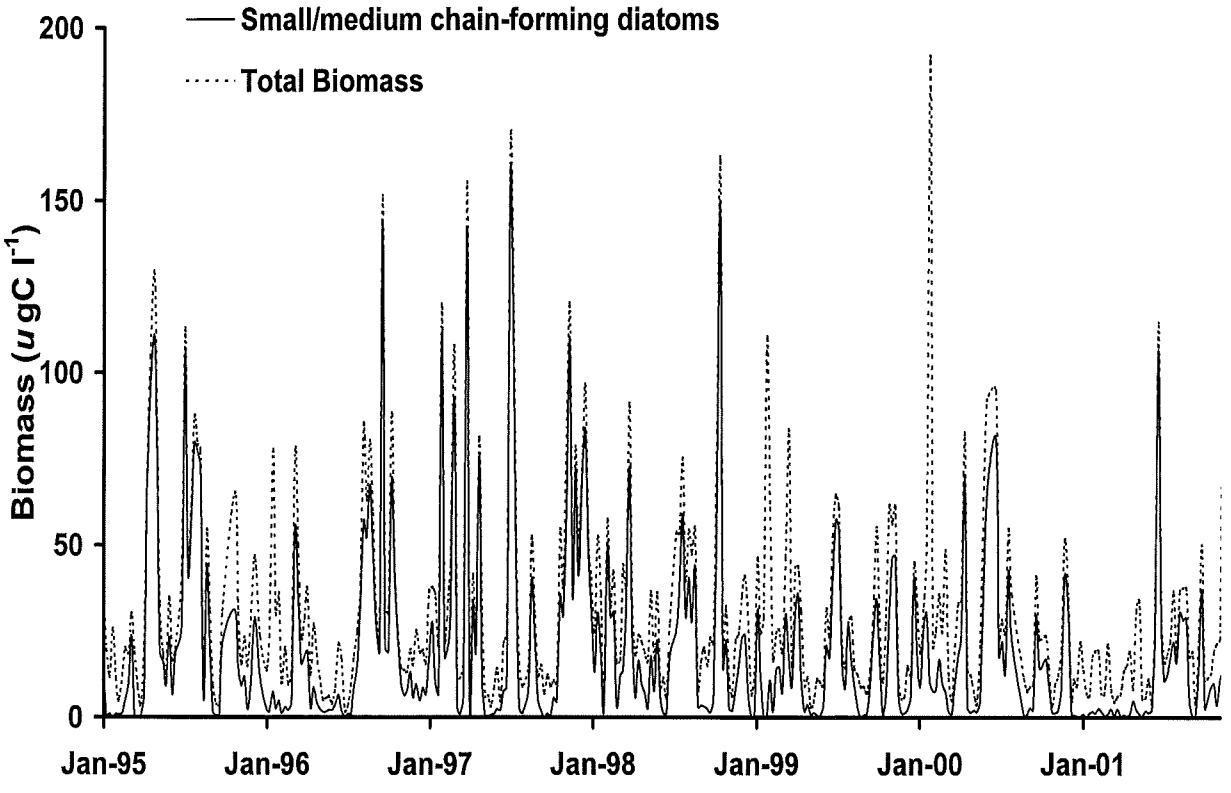

Figure 6.1. Total phytoplankton biomass (dashed line) and biomass of small/medium sized chain-forming diatom taxa (solid line) in Beatrix Bay between 1995 and 2002. Data courtesy of NIWA monitoring program. Data represent single weekly samples from site WB.

due to a fluctuating nutrient supply, caused by variable upwelling in Cook Strait and a constantly evolving, salinity-driven stratification. In contrast, dinoflagellates only tend to dominate Beatrix Bay phytoplankton biomass during summer. Periods of prolonged thermal stratification create a quasi-steady state that favours these slow-growing taxa (Cushing 1989).

Other coastal ecosystems that receive pulses of nutrient supply have a similar phytoplankton assemblage to Beatrix Bay. For example, the northern Adriatic Sea receives most of its inorganic nitrogen via riverine inputs, which are temporally variable (Carlsson and Graneli 1999; Aubry et al 2004). The phytoplankton assemblage is dominated most of the time by chain-forming diatom taxa such as *Chaetoceros* sp., *Pseudonitzschia* sp., *Skeletonema* sp., and *Thalassiosira* sp (Aubry et al. 2004). Dinoflagellates are only present in significant abundance during a two-month period in mid-summer when the water column is thermally stratified and nutrients are exhausted from the upper water layer (Carlsson and Graneli 1999; Aubry et al. 2004). The northern Gulf of Mexico receives frequent riverine nitrate enrichments via the Mississippi and Atchafayala Rivers, and contains a phytoplankton community dominated by chain-forming diatoms such as *Skeletonema* sp. and *Pseudonitzschia* sp. (Bode and Dortch 1996). As in Beatrix Bay, these taxa not only dominate bloom periods, but also are abundant throughout the year.

Beatrix Bay is an example of a coastal ecosystem in which anthropogenic nutrient inputs are low (Table 1.1). The major source of dissolved inorganic nitrogen to the upper water column is the advection of oceanic water into Beatrix Bay via the main Pelorus channel (Gibbs et al. 1992, 2002; Dupra 2000). Phytoplankton in coastal ecosystems that receive large amounts of anthropogenic nutrient inputs may rarely be nutrient-limited. Light and temperature are generally considered the key factors structuring phytoplankton community dynamics in these coastal ecosystems. For example, San Francisco Bay is nutrient-rich, receiving agricultural inputs and sewage inputs from a local population of 8 million people (Cloern 1996). Phytoplankton growth in this bay is primarily considered to be a function of light availability, not nutrient availability (Cloern et al. 1985). The lower Hudson estuary in the vicinity of New York City receives 6.1 x 10^7 kg N yr^{-1} through domestic wastes, and hence nitrogen is never limiting to phytoplankton growth (Malone 1977). Instead, phytoplankton growth is regulated by light and temperature, increasing during summer when irradiance and temperature increase (Malone 1977).

6.4 INTERANNUAL CLIMATIC FORCING

Interannual variability in Beatrix Bay phytoplankton dynamics in summer appears to be associated with large-scale climatic forcing driven by the El Niño-Southern Oscillation (ENSO) phenomenon (Fig. 5.13). Interannual ENSO-related changes in wind-driven upwelling in Cook Strait alter diatom-dinoflagellate dynamics during summer in Beatrix Bay. During 'normal conditions' (when ENSO is neutral or in a La Niña phase), dinoflagellate biomass tends to be high in Beatrix Bay during summer. During strong El Niño phases in summer, increased upwelling in Cook Strait results in higher nitrate availability to Beatrix Bay phytoplankton and a higher than normal diatom biomass and lower than normal dinoflagellate biomass (Fig. 5.13).

ENSO has been linked to changes in primary productivity in other studies. El Niño conditions are associated with a shutting down of upwelling and a decrease in phytoplankton biomass in the equatorial Pacific near New Caledonia (Blanchot et al. 1992) and off the coast of South America (Chavez et al. 1999). Sea-viewing Wide Field-of-view Sensor (SeaWiFS) ocean colour satellite imagery has revealed that phytoplankton responses to ENSO are global in extent (Behrenfeld et al. 2001).

ENSO-induced changes in primary productivity affect trophic levels up through the food chain, including populations of zooplankton (Barber and Chavez 1983), small schoolfish (Tsai et al. 1987), pelagic fish (Livingston 2000), seabirds (Boersma 1978; Tershy et al. 1991), and cetaceans (Tershy et al. 1991). These trophic effects may be particularly significant in Beatrix Bay, which is the site of intensive aquaculture of the green-lipped mussel *Perna canaliculus*. The relationship between ENSO and phytoplankton dynamics in Beatrix Bay could have major implications for mussel farming within the bay. This is because a change in the diatom-dinoflagellate communities at the base of the food chain can have a large impact on higher trophic levels (Cushing 1989; Mann 1993). Dinoflagellates are considered three to five times more nutritionally valuable per unit volume than diatoms (Chan 1980; Hitchcock 1982). *Perna canaliculus* has higher assimilation efficiency when fed on dinoflagellate cultures than diatom cultures (J. Ren, unpublished data). Therefore, changes in ENSO could affect food quality to mussels in Beatrix Bay. However, because most toxic phytoplankton taxa in marine systems are dinoflagellates (Paerl 1988), the risk of harmful algal blooms occurring in Pelorus Sound may be greater during La Niña phases. Further

research is needed into the relationship between ENSO and mussel condition in Beatrix Bay. ENSO might become a predictive tool for the management of mussel farms in Pelorus Sound. Mussel density within farms and harvesting could be adjusted depending on the predicted phytoplankton biomass and food quality.

6.5 LIGHT

Mean daily solar irradiance was low during winter and high during summer, ranging from 20 kJ m^{-2} in June 2002 to 1420 kJ m^{-2} in December 2002 (Fig. 3.5b). The average critical depth of the Beatrix Bay water column ranges from 10 m in mid-winter to 60 m in mid-summer (Gall et al. 2000). Therefore, there is the potential for phytoplankton that are mixed below 10 m depth in mid-winter to be light-limited. The enclosure experiments of Chapter 3 indicated that light limitation of phytoplankton was the most important factor determining biomass during mid-winter (Fig. 3.10, Table 3.8). Gibbs and Vant (1997) attributed the low growth rates of Beatrix Bay phytoplankton during winter, a time when nitrate is abundant, to light limitation. Response to light levels does not appear to be as important as nitrate levels in determining phytoplankton community composition in Beatrix Bay. Taxa that had a significant overall reduction in biovolume in the experimental shading treatments were both abundant (e.g. *Chaetoceros* sp.) and rare (e.g. *Coscinodiscus* sp.) in Beatrix Bay between 1994 and 2002 (Table 3.15; Fig. 3.17).

6.6 SPATIAL VARIABILITY

Advection of phytoplankton into Beatrix Bay plays a major role in the spatial variation of phytoplankton across the bay throughout the year. The main channel outside the bay generally has higher nitrate concentrations year round (Fig. 4.6), and is usually well-mixed due to high tidal velocities (Gibbs et al 2002; Stevens 2003). The western side of Beatrix Bay has higher hydrodynamic exchange with the main channel than the eastern side of the bay (Fig. 4.2, 4.3, 4.7). During summer, when nitrate is limiting to phytoplankton in Beatrix Bay, biomass tends to be higher in the main channel where there is more nitrate (Fig. 4.9). This biomass is also comprised of a greater percentage of diatoms, which generally have a higher nitrate requirement than dinoflagellates. Advection of this high biomass water into western Beatrix Bay results in a higher phytoplankton biomass, comprised of a greater proportion of diatoms, in western Beatrix Bay than in eastern Beatrix Bay at this time. During winter, phytoplankton in the well-mixed main channel tends to be light-limited, and consequently

biomass is lower there (Fig. 4.9). Advection of this low biomass water into western Beatrix Bay dilutes the phytoplankton concentration, resulting in lower biomass in comparison with eastern Beatrix Bay at this time of the year. Phytoplankton composition tends to be similar across Beatrix Bay during this period.

6.7 TEMPERATURE

Growth rates of marine phytoplankton tend to decrease with decreasing temperature (Parsons et al. 1984; Ahlgren 1987; Montagnes and Franklin 2001). Temperature may be an important factor in coastal ecosystems that undergo large seasonal temperature changes. For example, in the northwest Adriatic Sea, temperature declines from 25 °C in late summer to 6 °C in winter/autumn (Aubry et al. 2004). It is thought that low phytoplankton abundance in the Adriatic Sea in autumn is due to low temperature at this time in conjunction with low irradiance (Aubry et al. 2004). Temperature in the upper water column in Beatrix Bay varies between 10 °C and 20 °C throughout the year, reflecting seasonal variation in solar irradiance (Appendix 6; Gibbs and Vant 1997; Gall et al. 2000; Gibbs et al. 2002). The temperature range in Beatrix Bay is within the critical growth range of most phytoplankton taxa and is unlikely to have a major impact on phytoplankton community composition (Jitts et al. 1964; Parsons et al. 1978; Montagnes and Franklin 2001).

6.8 GRAZING

This thesis was primarily concerned with the role of bottom-up processes in structuring the phytoplankton community. Traditionally in marine pelagic ecology more importance has been accorded to bottom-up resource acquisition forces over top-down predation in structuring marine pelagic systems (Verity and Smetacek 1996). Bottom-up processes determine the potential for phytoplankton blooms to occur. Whether this potential is realised is often dependent on grazing pressure (top-down control).

Most bivalve grazers have a much lower metabolic rate than zooplankton grazers and have the capacity to live on stored reserves, enabling them to survive periods of low food concentration (Prins et al. 1998). Consequently, bivalve grazers will already be present when a phytoplankton bloom develops, whereas zooplankton development lags behind phytoplankton bloom development. This has led to the hypothesis that top-down control by bivalves will be more efficient than control by zooplankton (Prins et al. 1998).

In Beatrix Bay, localised effects of mussel grazing on phytoplankton have been found within farms. Ogilvie et al. (2000) found depletion of phytoplankton occurred within mussel farms during winter. The mussels did not have a significant influence on taxonomic richness of the phytoplankton, and appeared to remove cells in an unselective manner (Ogilvie 2000). During summer, phytoplankton levels were significantly higher within mussel farms (Ogilvie et al. 2000). This localised increase was attributed to mussels, which excrete dissolved inorganic nitrogen, supplementing low ambient nitrogen concentrations.

Microzooplankton grazing can potentially have a significant effect on phytoplankton biomass throughout the year (Gallegos et al. 1996). There was no seasonal cycle apparent in ciliate biovolume in Beatrix Bay (Fig. 3.8a). Biovolume ranged between 50 000 and 200 000 μm^3 ml^{-1} during all field sampling trips except for March 2002, when ciliate biovolume was 464 000 μm^3 ml^{-1} (Fig. 3.8a). A ciliate grazing experiment was performed during February 2003, when ciliate biovolume was 93 600 μm^3 ml^{-1}. At this time, ciliate grazing rate was 55% of nanophytoplankton population growth rate and 45% of picophytoplankton population growth rate (Table 3.2). This demonstrates that there is potential for high ciliate abundance to have a significant grazing impact on smaller phytoplankton size classes. It appears that the lack of a phytoplankton growth response to nutrient addition during an enclosure experiment in March 2002 (Fig. 3.9, 3.10), when ambient nitrate was at limiting levels to phytoplankton, was due to a high biomass of ciliates present at the time grazing any excess phytoplankton production. Previous studies in the South Island of New Zealand demonstrated that ciliates could potentially graze over 90% of small phytoplankton biomass (James et al. 1996; James and Hall 1998).

Macrozooplankton (>250 μm) can have a large grazing effect on Beatrix Bay phytoplankton (Gall et al. 2000). Macrozooplankton biomass in Beatrix Bay tends to be high during summer and low during winter (Gall et al. 2000). Furthermore, grazing rates tend to increase with temperature (Raymont 1983; Froneman 2001). Therefore macrozooplankton grazing intensity is highest during summer in Beatrix Bay, and it is thought that low macrozooplankton grazing intensity during the winter months contribute to the high phytoplankton biomass present at the time (Gall et al. 2000).

6.9 BEATRIX BAY PHYTOPLANKTON MODEL

Research in this thesis advances the understanding of the factors structuring phytoplankton community dynamics in Beatrix Bay. The links between this study and current conceptual models of Beatrix Bay are shown for summer (Fig. 6.2) and winter (Fig. 6.3). Summer is characterised by stable thermal stratification and high light levels. Phytoplankton under these high light conditions have a high demand for nitrate, and consequently nitrate concentrations in the upper water column become exhausted. These conditions favour motile dinoflagellates, which can exploit both the overlying euphotic zone and the underlying nutrient-rich waters. Diatom taxa have higher nitrate requirements and tend to sink out of stratified water columns. Biomass in western Beatrix Bay tends to be higher than eastern Beatrix Bay and comprises a greater proportion of diatoms. This is due to advection of these cells into western Beatrix Bay from the main Pelorus channel, where nitrate concentration is higher. ENSO can impact on phytoplankton dynamics in Beatrix Bay during summer, with El Niño events favouring diatom growth due to an increase in upwelling of nitrate-rich water in Cook Strait and its subsequent advection into Beatrix Bay. Macrozooplankton biomass and grazing is highest at this time, and macrozooplankton grazers may have a significant effect on phytoplankton biomass.

During winter (Fig. 6.3), stratification is salinity-driven and related to the variable freshwater flux from the Pelorus River. Light levels are low and may be limiting to phytoplankton growth when mixed deeply in the water column. The nitrate demand of phytoplankton is lower under the low light conditions, and therefore nitrate levels in the upper water column are non-limiting. Phytoplankton biomass is dominated by chain-forming diatom taxa such as *Chaetoceros* sp., *Pseudonitzschia* sp., and *Skeletonema* sp, which thrive under non-limiting nutrient conditions. Biomass tends to be higher in eastern Beatrix Bay than western Beatrix Bay. This is due to low biomass water being advected in to western Beatrix Bay from the main Pelorus channel, where water column mixing is deep and light limitation of phytoplankton severe. Macrozooplankton biomass is low at this time and macrozooplankton are unlikely to have a major top-down effect on phytoplankton biomass.

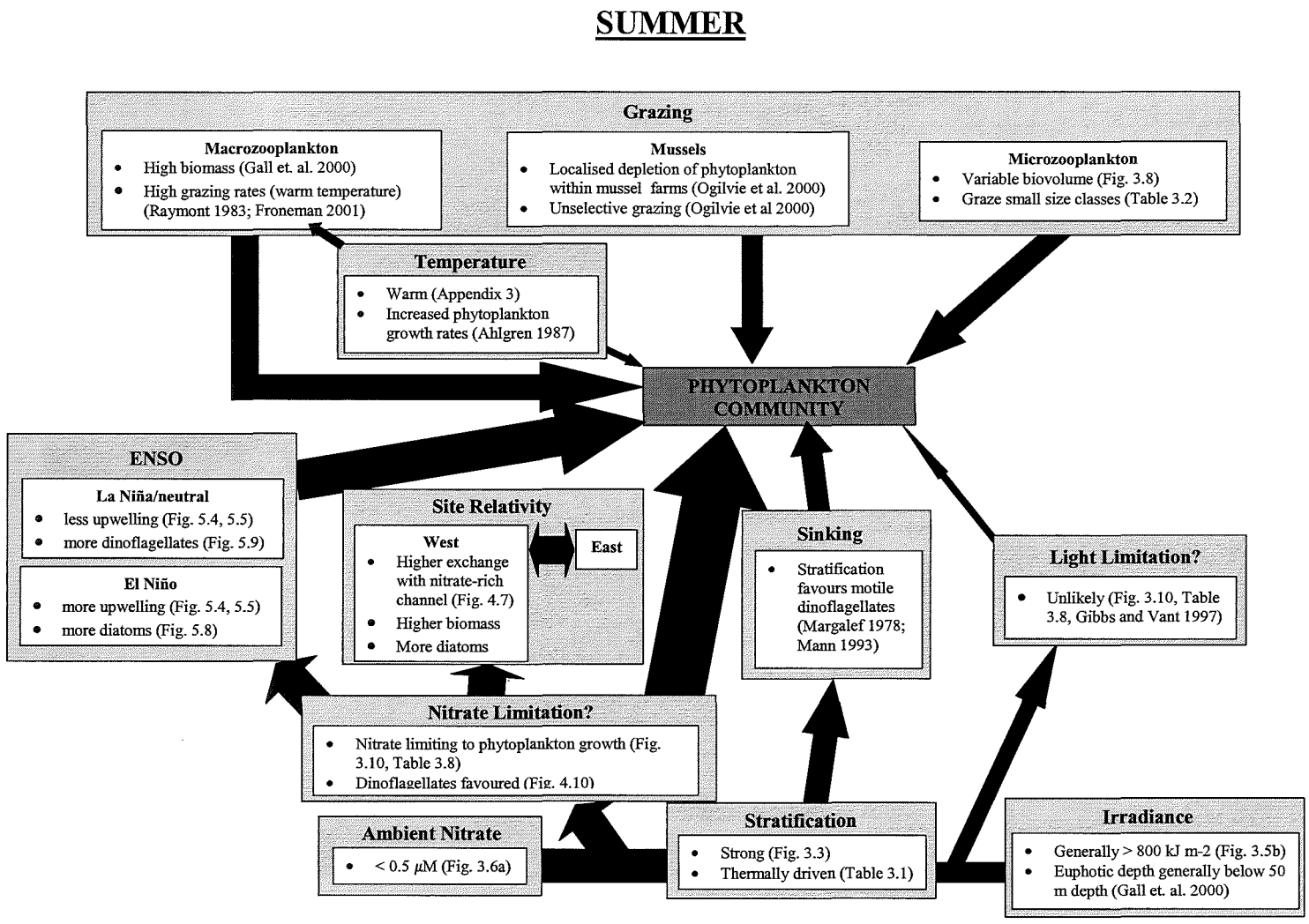

Figure 6.2. Effects flow diagram summarising the processes structuring the autotrophic phytoplankton community in Beatrix Bay during summer. The estimated strength of each process is indicated by the thickness of the arrows.

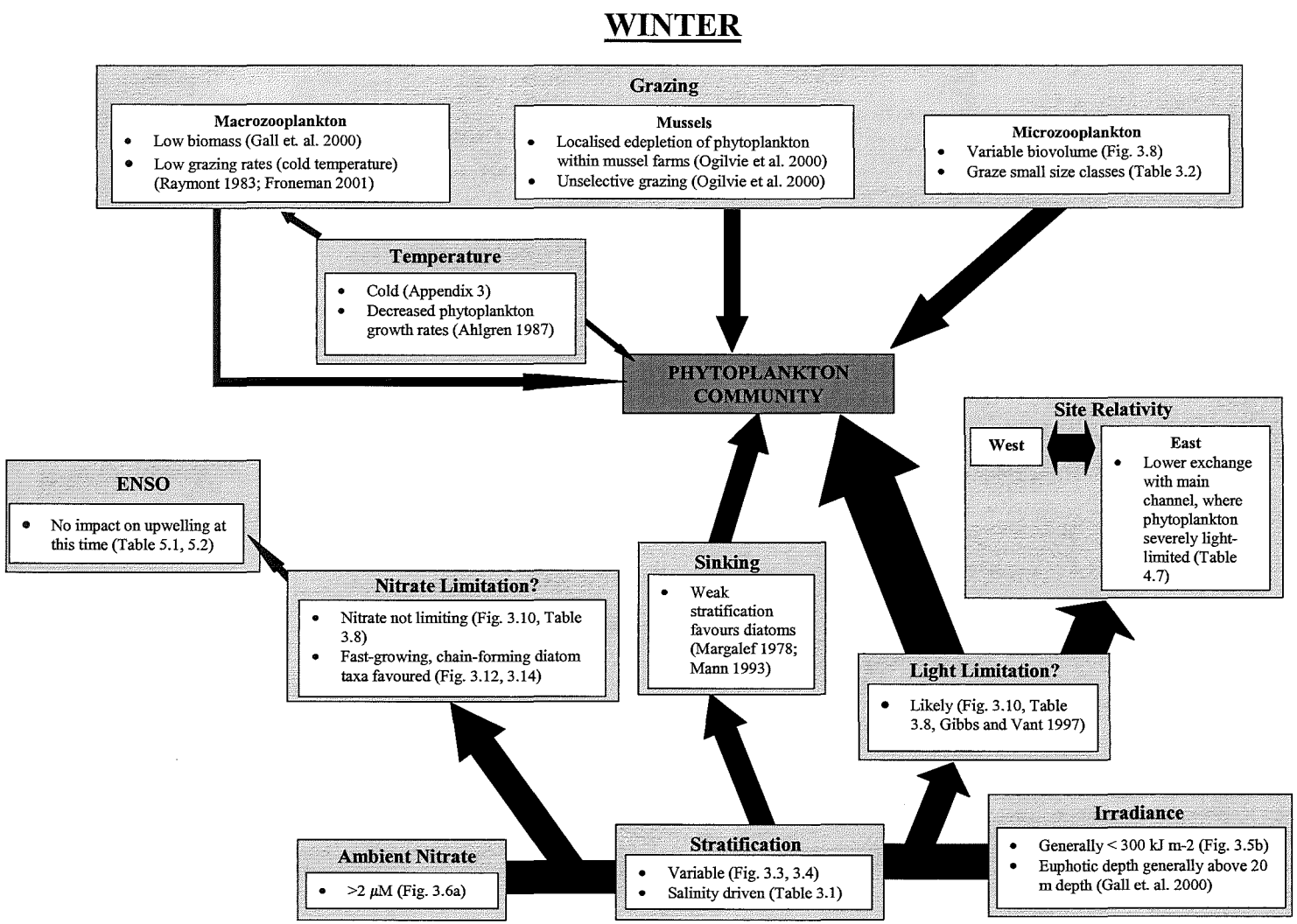

Figure 6.3. Effects flow diagram summarising the processes structuring the autotrophic phytoplankton community in Beatrix Bay during winter. The estimated importance of each process is indicated by the thickness of the arrows.

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APPENDIX 1. List of Phytoplankton Taxa and Biovolumes

Phytoplankton taxa and mean individual cell volumes used in phytoplankton analyses.

PHYTOPLANKTON TAXA	MEAN CELL VOLUME (μm^3)		
Dinoflagellates			
Ampidinium sp.	5666	*Gyrodinium* sp. (s)	6394
Cachonina sp.	3175	*Gyrodinium* sp. (l)	26394
Ceratium furca	72046	*Heterocapsa triquetra*	10648
Ceratium fusus	15625	*Heterocapsa* sp.	3550
Ceratium tripos	15625	*Oxytoxum* sp.	1616
Ceratium sp.	20000	*Peridinium* sp.	21952
Dinophysis sp.	30300	*Polykrikos* sp.	60000
Diplopsalis sp.	15000	*Prorocentrum* sp.	31000
Fragilidium sp.	3500	*Protoperidinium* sp. (s)	5000
Gambierdiscus sp.	3500	*Protoperidinium* sp. (l)	34000
Gonyaulax sp.	7000	*Pyrocystis lunula*	20000
Gymnodinium sp. (s)	1100	*Scrippsiella trochoidea*	5813
Gymnodinium sp. (m)	2000	*Torodinium* sp.	9000
Gymnodinium sp. (l)	7000		
Diatoms			
Actinoptychus sp.	200	*Leptocylindricus danicus*	2050
Asterionellopsis sp.	1000	*Leptocylindricus mediterraneous*	2000
Asteromphalus sp.	2200	*Leptocylindricus minimus*	500
Bacillaria sp.	300	*Melosira* sp.	5000
Bacteriastrum sp.	20	*Navicula* sp.	2000
Biddulphia sp.	15000	*Navicula membranacea*	10000
Cerataulina sp.	5000	*Nitzschia closterium*	90
Cerataulus sp.	5000	*Nitzschia longissima*	175
Chaetoceros sp. (s)	500	*Nitzschia* sp.	220
Chaetoceros sp. (l)	1820	*Odontella* sp.	10000
Cocconeis sp.	2000	*Paralia* sp.	400
Corethron sp.	5000	*Pleurosigma* sp.	15410
Coscinodiscus sp.	200000	*Pseudonitzschia* sp.	3000
Denticulopsis sp.	2000	*Rhizosolenia imbricata*	6000
Diploneis sp.	600	*Rhizosolenia setigera*	4375
Ditylum brightwelli	42000	*Rhizosolenia styliformus*	6000
Eucampia zoodiacus	3000	*Rhizosolenia* sp.	6000
Eucampia sp.	3000	*Skeletonema* sp.	525
Grammatophora sp.	100	*Stephanopyxis* sp.	60000
Guinnardia flaccida	9000	*Thalassiosira* sp.	5000
Guinnardia sp.	5000	*Thalassionema nitzschiodies*	1512
Gyrosigma sp.	16000	*Thallassiothrix* sp.	1200
Hemiaulus sp.	3000	*Toxarium* sp.	1300
Lauderia annulata	3000		
Other			
Dictyocha sp.	200	*Heterosigma akash*	40
Eutraptiella sp.	6000	Small flagellates	50
Fibrocapsa japonic	100		
Ciliates			
Ciliates (s)	10000	*Mesodinium rubrum*	50000
Ciliates (m)	30000	Tintinids	65000
Ciliates (l)	60000		

APPENDIX 2. Mesocosm Experiment

Comparison of Nutrient-Enhanced Phytoplankton Growth in 25 000 l Mesocosms and 12 l Cubitainers

Introduction

The size of the enclosure is an important aspect in the design of aquatic enclosure experiments. An increase in enclosure size has both beneficial and detrimental effects. Larger size leads to a decrease in wall effects, and hence larger enclosures are likely to be more 'natural'. However, larger size also increases the structural heterogeneity of the enclosed water, decreasing the chance of duplicating the system (Gamble and Davis 1982). During this study, nutrient and light manipulation experiments were done using 12 l cubitainers. The aim of this experiment was to examine whether results attained in 12 l cubitainers mirrored what occurred in much larger-scale, 25 000 l mesocosms.

Methods

There were two treatments: a control treatment and a nutrient addition treatment. There were three replicates of each treatment. In the nutrient addition treatment, a nutrient spike solution of N, P, and Si in deionised water was added. The nutrient spike was designed to raise the nutrient levels of N, P, and Si by approximately 5 μM, 0.3 μM, and 5 μM respectively (mirroring the Redfield ratio for ideal maximum growth of 16:1:16).

Each mesocosm consisted of a large, 8.5 m long polyethylene 'sock' attached to a 2 m diameter metal collar (Fig. 1). There were six mesocosms, each approximately 25 000 l in volume. They were lowered to 7 m depth and then raised by a winch, partially filling them. They were then attached to a mussel farm backbone and completely filled using a pump. The nutrient solution was then added to the nutrient treatments and given at least one hour to mix. Each mesocosm was mixed throughout the incubation period using air bubbles from a dive tank released at the bottom of the mesocosm. Each cubitainer was then filled from a different mesocosm to ensure starting phytoplankton and nutrient levels were comparable. The cubitainers were attached to the mussel backbone at 4 m depth. A storm that hit the site after the second day of the experiment damaged most of the mesocosms, terminating the experiment prematurely.

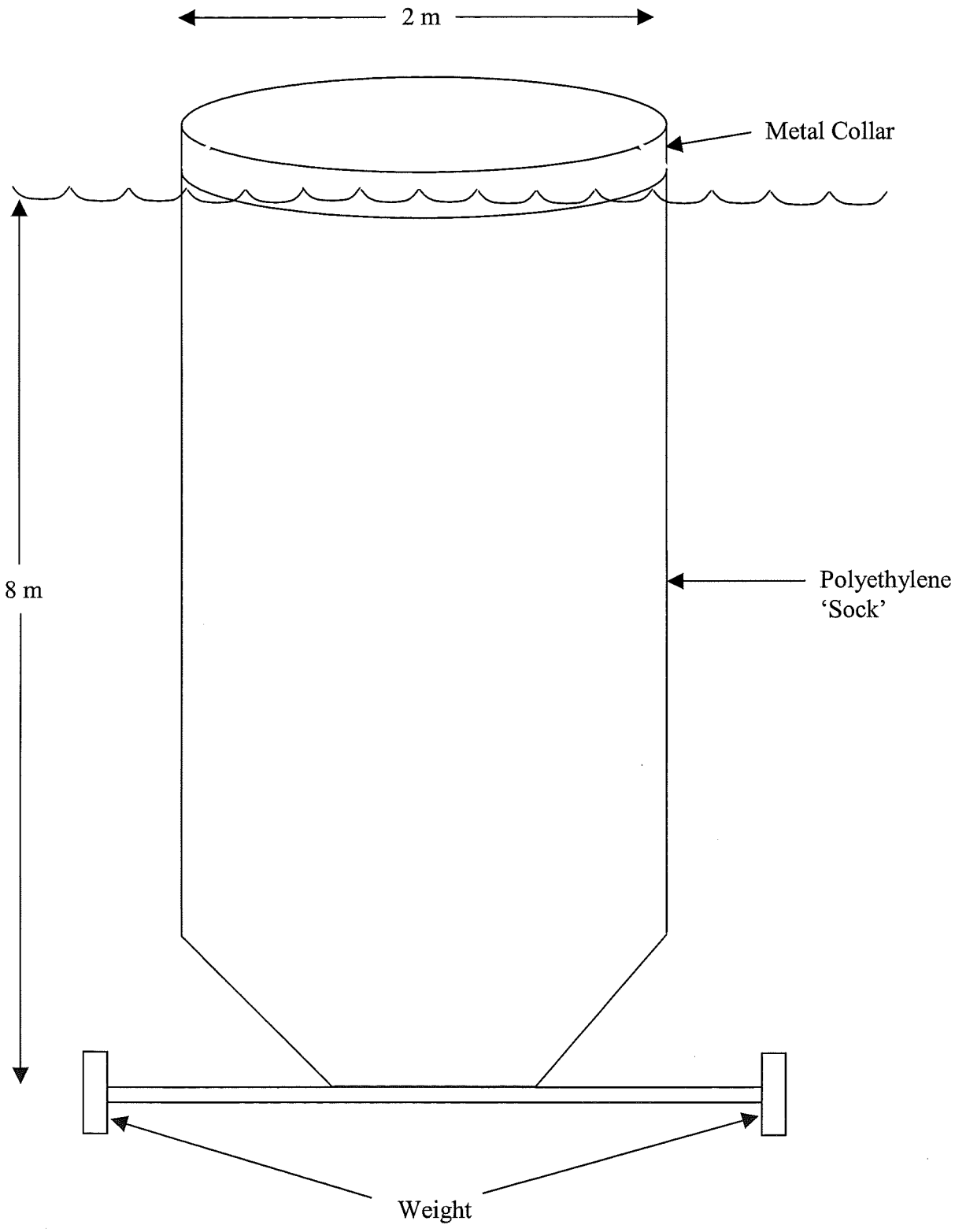


Figure 1. Mesocosm design. An 8 m long polyethylene 'sock' was attached to a 2 m diameter metal collar. The mesocosm 'sock' was weighted down at the bottom. The collars were attached to a mussel farm backbone.

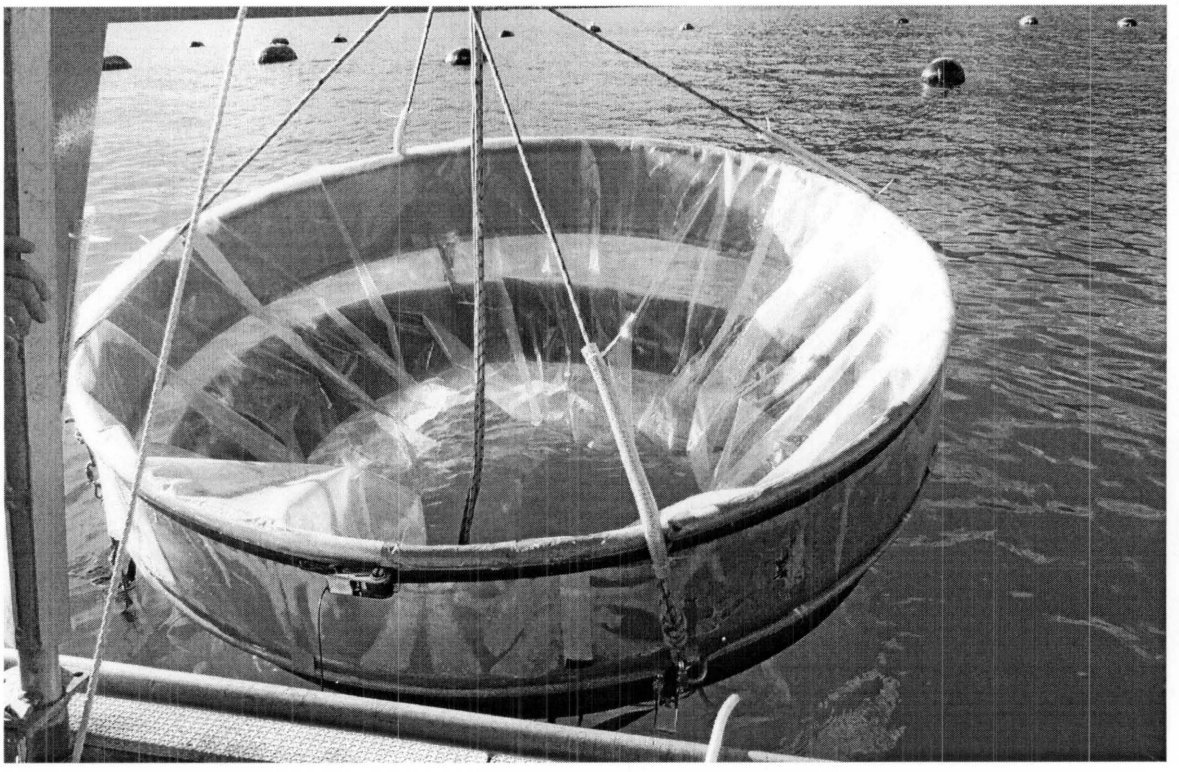


Figure 2. Mesocosm being lowered into the water column by winch.

Results and Discussion

There were similar levels of nitrate uptake by phytoplankton within the mesocosm and cubitainer enclosures during the experiment (Fig. 3, Table 1). Total phytoplankton growth and growth within each of the microphytoplankton, nanophytoplankton, and picophytoplankton size classes was similar within both enclosure types after 1 day (Fig. 4, Fig. 5, Table 2, Table 3).

This was a pilot experiment in which deployment and filling of the mesocosms was still being tested. Therefore, experimental protocols were not ideal. However, it did provide an insight into how relevant results from cubitainers are when compared with much larger scale systems. Increases in phytoplankton within the cubitainers mirrored what occurred in the mesocosms, albeit after only one day. How similar microcosm and larger scale results are over a longer term are yet to be determined.

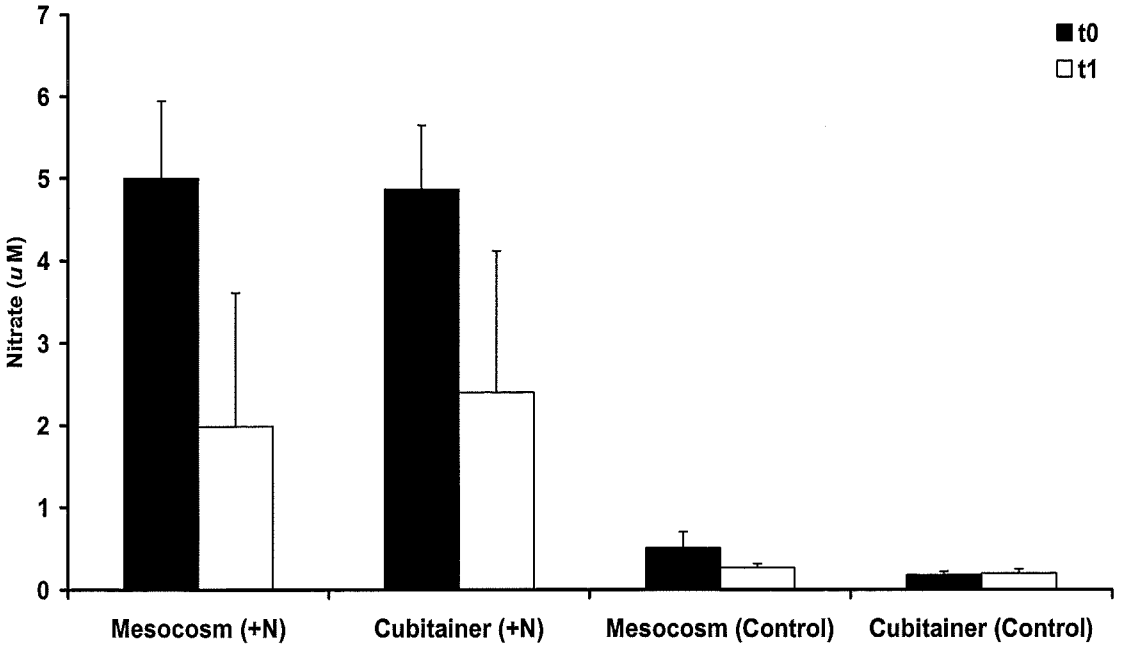


Figure 3. Average nitrate concentration in mesocosm and cubitainer enclosures during experiment. Treatments are added nutrients (+N) and control. Error bars are ± 1 SE.

Table 1. Summary of 3-way ANOVA. Independent factors are enclosure type, treatment and day. Dependent variable is nitrate concentration. Asterisks: *p<0.05.

Source	DF	MS	F	*p*
Enclosure Type (ET)	1	0.008	0.35	0.562
Treatment	1	0.897	38.87	0.000*
Day	1	0.147	6.35	0.023*
ET*Treatment	1	0.022	0.96	0.342
ET*Day	1	0.009	0.39	0.539
Treatment*Day	1	0.088	3.82	0.068
ET*Treatment*Day	1	0.001	0.03	0.865
Residual	16	0.023		

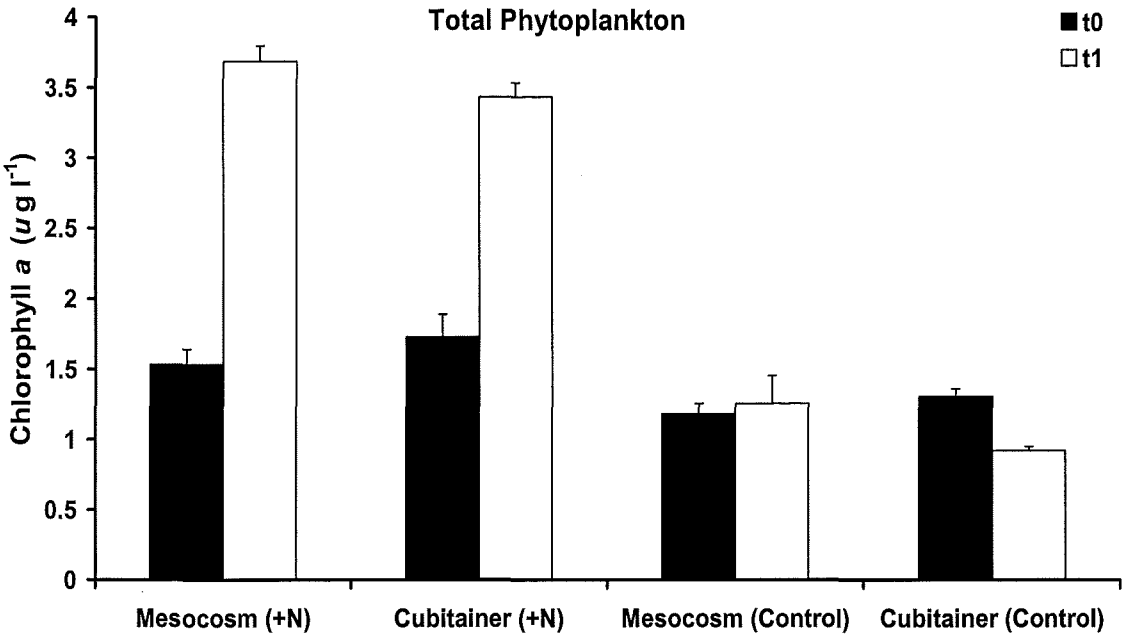


Figure 4. Average bulk chlorophyll *a* concentration in mesocosm and cubitainer enclosures during experiment. Treatments are added nutrients (+N) and control. Error bars are ± 1 SE.

Table 2. Summary of 3-way ANOVA. Independent factors are enclosure type, treatment and day. Dependent variable is bulk chlorophyll *a* concentration. Asterisks: *p<0.05.

Source	DF	MS	F	*p*
Enclosure Type (ET)	1	0.022	0.16	0.695
Treatment	1	17.272	127.11	0.000*
Day	1	4.717	34.72	0.000*
ET*Treatment	1	0.005	0.04	0.844
ET*Day	1	0.248	1.83	0.195
Treatment*Day	1	6.531	48.07	0.000*
ET*Treatment*Day	1	0.002	0.02	0.896
Residual	16	0.136		

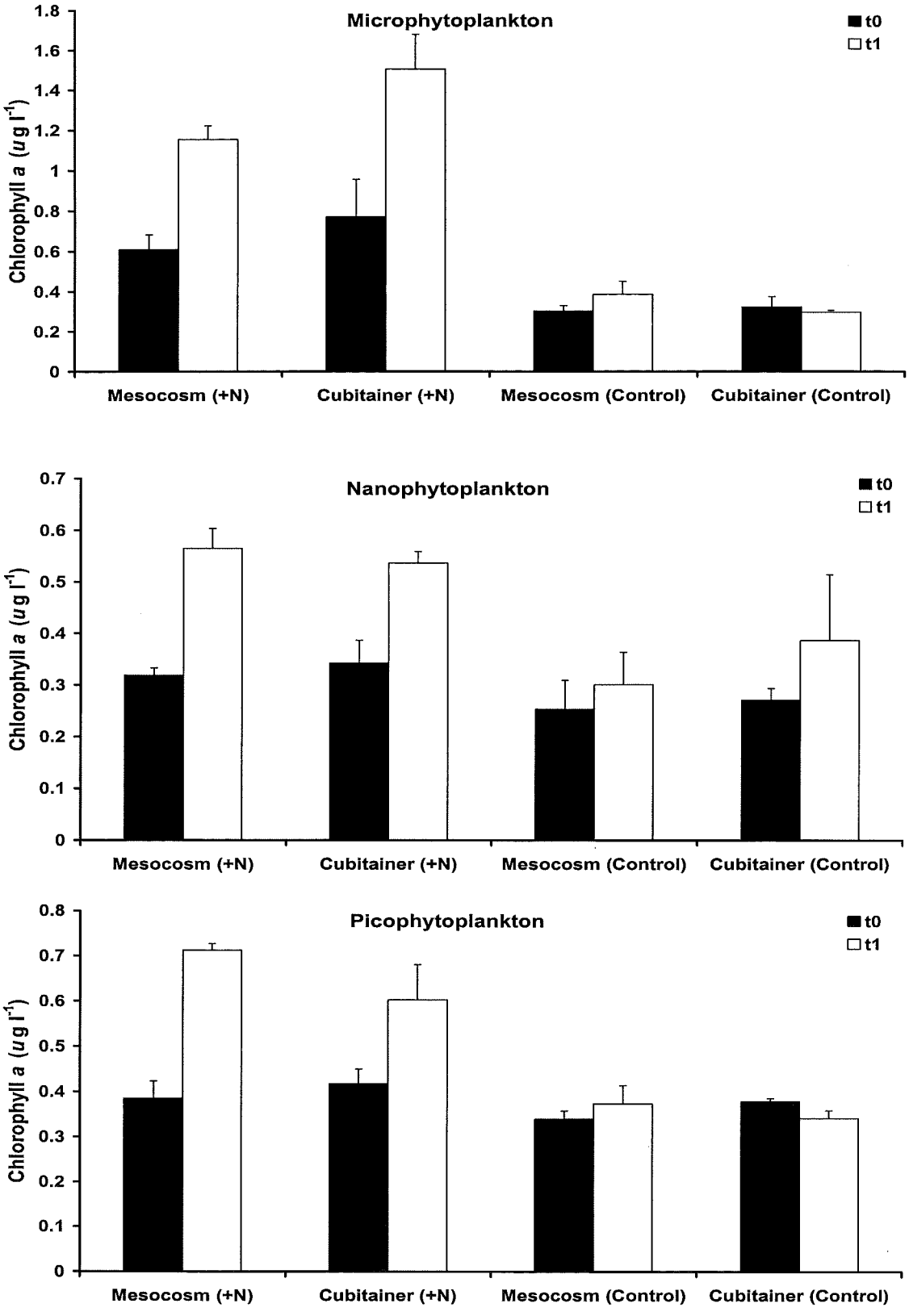


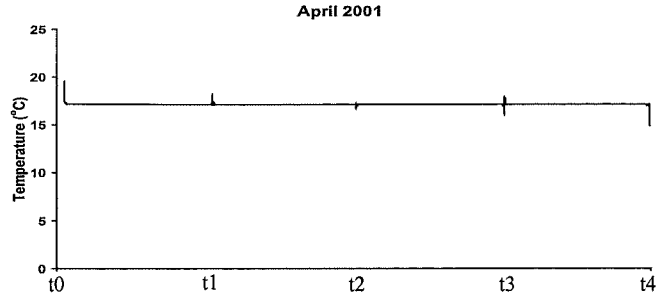
Figure 5. Average chlorophyll *a* concentration for microphytoplankton (>20 μm diameter), nanophytoplankton (2-20 μm diameter) and picophytoplankton (<2 μm diameter) size classes in mesocosm and cubitainer enclosures during experiment. Treatments are added nutrients (+N) and control. Error bars are ± 1 SE.

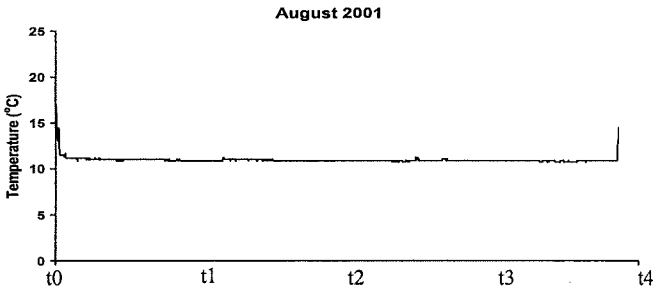
Table 3. Summary of 3-way ANOVA. Independent factors are enclosure type (E), treatment (T) and day (D). Dependent variables are microphytoplankton (>20 μm diameter), nanophytoplankton (2-20 μm diameter) and picophytoplankton (<2 μm diameter) chlorophyll *a* concentration. Asterisks: *p<0.05.

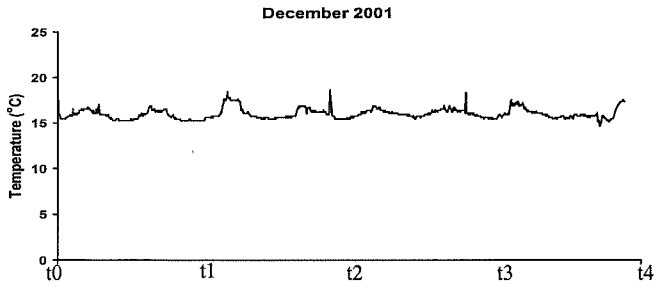
Source	DF	Microphytoplankton			Nanophytoplankton			Picophytoplankton		
		MS	F	*p*	MS	F	*p*	MS	F	*p*
E	1	0.079	2.00	0.177	0.002	0.18	0.678	0.002	0.20	0.661
T	1	3.540	90.04	0.000*	0.225	23.65	0.000*	0.337	33.65	0.000*
D	1	0.631	16.04	0.001*	0.118	12.36	0.003*	0.102	10.13	0.006*
E*T	1	0.119	3.01	0.102	0.002	0.23	0.636	0.002	0.25	0.627
E*D	1	0.005	0.12	0.730	0.000	0.00	0.949	0.014	1.39	0.255
T*D	1	0.596	15.16	0.001*	0.039	4.05	0.061	0.096	9.57	0.007*
E*T*D	1	0.026	0.66	0.430	0.003	0.34	0.566	0.003	0.31	0.583
Residual	16	0.039			0.010			0.010		

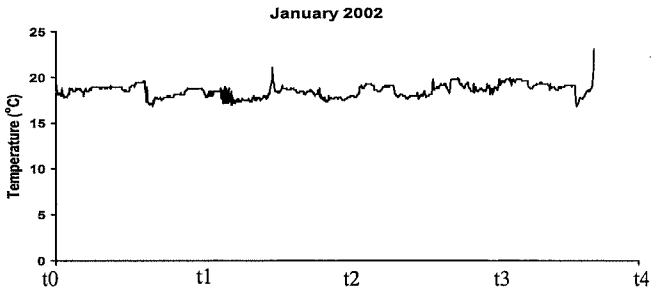
APPENDIX 3. Temperature Profiles

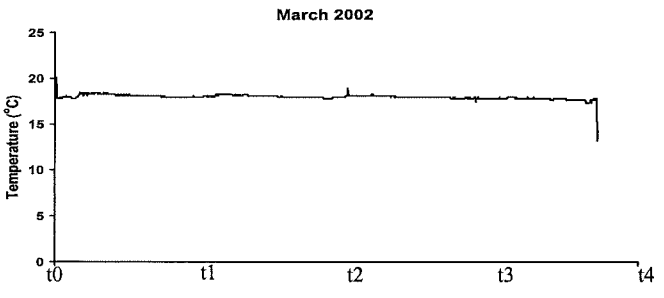
Temperature profiles at experimental incubation site during each enclosure experiment incubation, collected by an attached Onset Stowaway TidbiT® temperature logger.

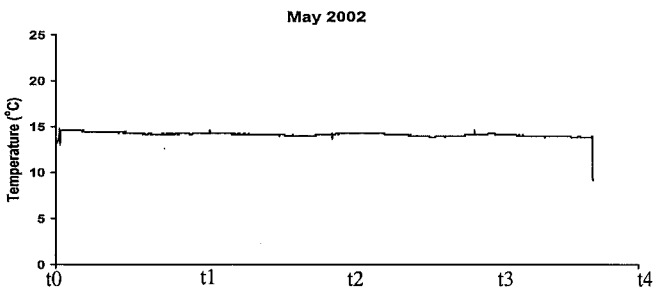


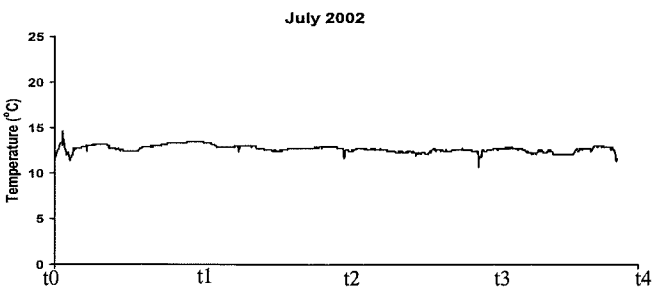


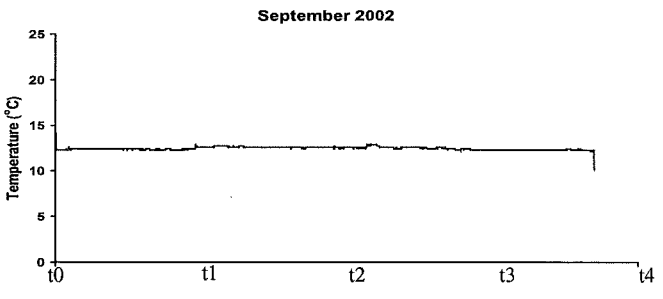


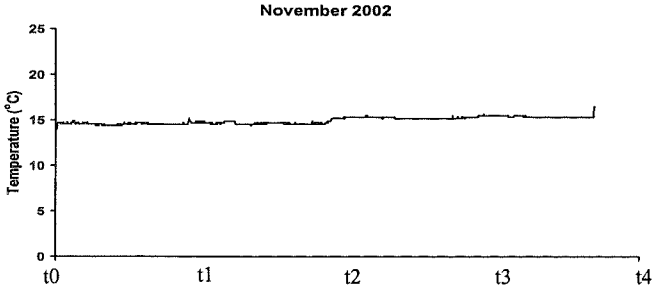


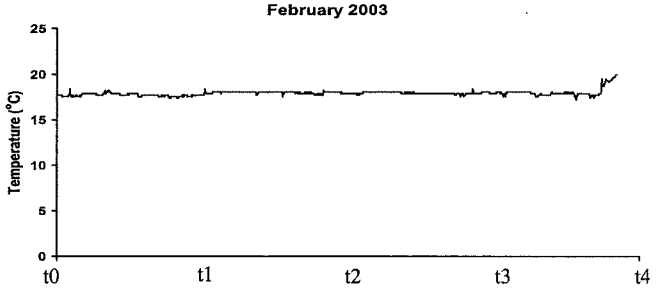












APPENDIX 4. Chapter 4 ANOVA Summary Results

Water Column Structure

Summary of one-way ANOVA. Independent factor is site. Dependent variable is density difference. Density difference calculated as density at 20 m depth minus density at 1 m depth. Asterisks: *($p<0.05$).

April

	df	MS	F	p
Site	1	0.00	0.42	0.551
Residual	6	0.00		

August

	df	MS	F	p
Site	1	0.01	0.16	0.705
Residual	6	0.03		

December

	df	MS	F	p
Site	2	1.13	1.87	0.210
Residual	9	0.61		

January

	df	MS	F	p
Site	2	0.01	0.20	0.826
Residual	9	0.03		

March

	df	MS	F	p
Site	2	0.05	6.56	0.017*
Residual	9	0.01		

May

	df	MS	F	p
Site	2	0.00	0.29	0.753
Residual	9	0.01		

July

	df	MS	F	p
Site	2	0.07	0.30	0.752
Residual	9	0.23		

September

	df	MS	F	p
Site	2	0.01	0.20	0.822
Residual	9	0.06		

November

	df	MS	F	p
Site	2	0.02	0.43	0.685
Residual	9	0.05		

February

	df	MS	F	p
Site	2	0.04	0.25	0.719
Residual	9	0.02		

Nitrate Concentration

Summary of two-way ANOVA. Independent factors are site and day. Dependent variable is nitrate concentration. Asterisks: *($p<0.05$).

April

	df	MS	F	p
Site	2	0.02	13.06	0.00*
Day	4	0.01	5.32	0.00*
Site*Day	8	0.01	8.95	0.00*
Residual	30	0.00		

August

	df	MS	F	p
Site	1	0.19	11.17	0.00*
Day	4	0.22	13.45	0.00*
Site*Day	4	0.03	1.88	0.15
Residual	20	0.02		

December

	df	MS	F	p
Site	2	0.92	0.51	0.60
Day	4	3.09	1.72	0.17
Site*Day	8	1.72	0.96	0.49
Residual	30	1.80		

January

	df	MS	F	p
Site	2	3.57	7.57	0.00*
Day	4	1.09	2.31	0.08
Site*Day	8	0.52	1.10	0.39
Residual	30	0.47		

March

	df	MS	F	p
Site	2	13.25	18.51	0.00*
Day	4	2.20	3.07	0.03*
Site*Day	8	4.80	6.70	0.00*
Residual	30	0.72		

May

	df	MS	F	p
Site	2	0.12	374.6	0.00*
Day	4	0.00	11.0	0.00*
Site*Day	8	0.00	14.9	0.00*
Residual	30	0.00		

July

	df	MS	F	p
Site	2	146.9	5.28	0.01*
Day	4	658.4	23.67	0.00*
Site*Day	8	84.20	3.03	0.01*
Residual	30	27.80		

September

	df	MS	F	p
Site	2	4.89	3.36	0.05
Day	4	6.01	4.12	0.02*
Site*Day	8	2.68	1.84	0.13
Residual	30	1.46		

November

	df	MS	F	p
Site	2	0.23	2.80	0.08
Day	4	0.91	10.94	0.00*
Site*Day	8	0.04	0.52	0.83
Residual	30	0.08		

February

	df	MS	F	p
Site	2	0.28	0.18	0.84
Day	4	2.29	1.47	0.25
Site*Day	8	1.46	0.93	0.49
Residual	30	1.56		

Chlorophyll *a* Concentration

Summary of two-way ANOVA. Independent factors are site and day. Dependent variable is Chlorophyll *a* concentration. Asterisks: *($p<0.05$).

April

	df	MS	F	p
Site	2	0.04	4.54	0.02*
Day	3	0.27	31.56	0.00*
Site*Day	6	0.04	5.21	0.00*
Residual	24	0.01		

August

	df	MS	F	p
Site	1	0.18	68.98	0.00*
Day	3	0.05	18.73	0.00*
Site*Day	3	0.00	1.36	0.29
Residual	16	0.00		

December

	df	MS	F	p
Site	2	0.19	17.61	0.00*
Day	3	0.05	4.74	0.01*
Site*Day	6	0.06	5.58	0.00*
Residual	24	0.01		

January

	df	MS	F	p
Site	2	0.05	24.17	0.00*
Day	3	0.03	15.17	0.00*
Site*Day	6	0.01	3.74	0.01*
Residual	24	0.00		

March

	df	MS	F	p
Site	2	0.33	442.79	0.00*
Day	3	0.10	135.41	0.00*
Site*Day	6	0.03	34.85	0.00*
Residual	24	0.00		

May

	df	MS	F	p
Site	2	0.02	42.10	0.00*
Day	3	0.01	16.99	0.00*
Site*Day	6	0.00	2.62	0.04*
Residual	24	0.00		

July

	df	MS	F	p
Site	2	0.00	0.78	0.47
Day	3	0.28	100.44	0.00*
Site*Day	6	0.01	2.92	0.03*
Residual	24	0.00		

September

	df	MS	F	p
Site	2	0.03	226.7	0.00*
Day	3	0.00	9.7	0.00*
Site*Day	6	0.00	18.1	0.00*
Residual	24	0.00		

November

	df	MS	F	p
Site	2	0.00	2.41	0.11
Day	3	0.01	14.34	0.00*
Site*Day	6	0.00	1.70	0.17
Residual	24	0.00		

February

	df	MS	F	p
Site	2	0.17	148.40	0.00*
Day	3	0.06	51.25	0.00*
Site*Day	6	0.01	10.51	0.00*
Residual	24	0.00		

Nitrate-Addition Experiment

Summary of two-way ANOVA. Independent factors are site and treatment. Dependent variable is change in chlorophyll *a* concentration during the experiment. Asterisks: *($p<0.05$).

April

	df	MS	F	p
Site	1	0.05	4.26	0.02*
Treatment	1	1.45	118.52	0.00*
Site*Treatment	1	0.01	1.04	0.00*
Residual	8	0.01		

August

	df	MS	F	p
Site	1	1.40	38.11	0.00*
Treatment	3	37.96	1033.54	0.00*
Site*Treatment	3	0.89	24.33	0.00*
Residual	16	0.04		

December

	df	MS	F	p
Site	1	0.72	9.65	0.01*
Treatment	1	2.63	35.36	0.00*
Site*Treatment	1	0.25	3.32	0.11
Residual	8	0.07		

January

	df	MS	F	p
Site	1	0.01	0.47	0.51
Treatment	1	0.70	50.33	0.00*
Site*Treatment	1	0.03	2.03	0.19
Residual	8	0.01		

March

	df	MS	F	p
Site	1	0.11	0.11	0.75
Treatment	1	1.77	1.82	0.21
Site*Treatment	1	0.10	0.10	0.76
Residual	8	0.98		

May

	df	MS	F	p
Site	1	20.70	13.73	0.01*
Treatment	1	52.57	34.87	0.00*
Site*Treatment	1	0.12	0.08	0.79
Residual	8	1.51		

July

	df	MS	F	p
Site	1	0.26	1.88	0.21
Treatment	1	0.01	0.05	0.83
Site*Treatment	1	0.00	0.00	0.99
Residual	8	0.14		

September

	df	MS	F	p
Site	1	1.17	30.73	0.00*
Treatment	1	16.22	424.72	0.00*
Site*Treatment	1	0.68	17.78	0.00*
Residual	8	0.04		

November

	df	MS	F	p
Site	1	0.00	0.01	0.92
Treatment	1	9.67	46.66	0.00*
Site*Treatment	1	0.01	0.05	0.83
Residual	8	0.21		

February

	df	MS	F	p
Site	1	7.93	55.13	0.00*
Treatment	1	23.64	164.41	0.00*
Site*Treatment	1	4.74	32.97	0.00*
Residual	8	0.14		

APPENDIX 5. Taxa used in Canonical Correspondence Analysis

Group	Taxon
Diatom	*Cerataulina* sp.
	Chaetoceros sp.
	Coscinodiscus sp.
	Ditylum brightwelli
	Eucampia sp.
	Guinnardia sp.
	Lauderia sp.
	Leptocylindricus sp.
	Navicula sp.
	Pseudonitzschia sp.
	Rhizosolenia sp.
	Skeletonema sp.
	Thalassionema sp.
	Thalassiosira sp.
Dinoflagellate	*Ceratium* sp.
	Dinophysis sp.
	Diplopsalis sp.
	Gymnodinium sp.
	Gyrodinium sp.
	Heterocapsa sp.
	Peridinium sp.
	Prorocentrum sp.
	Protoperidinium sp.
	Scrippsiella trochoidea
Autotrophic Ciliate	*Mesodinium rubrum*

APPENDIX 6. Beatrix Bay Water Temperature

Average water temperature at West Beatrix site at 5 m depth. Water temperatures measured by Ocean Sensors Model OS200 APV profiler on five successive days. Error bars ± 1 SE.